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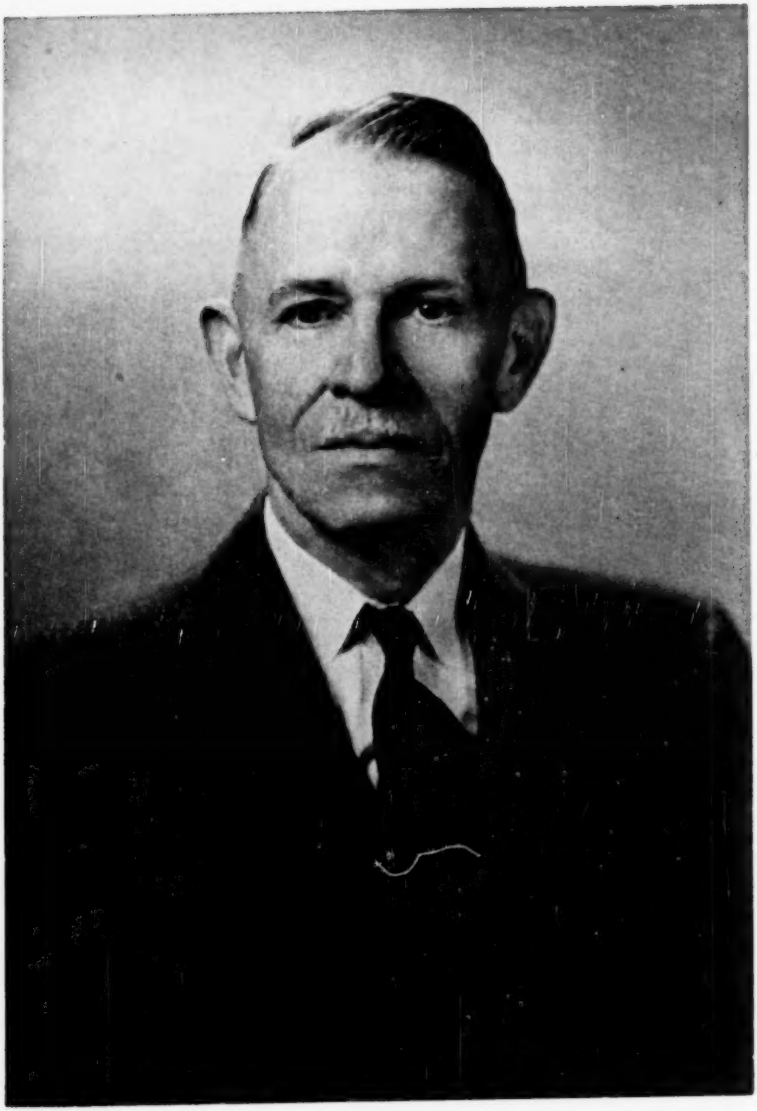

JULIAN H. MILLER, PRESIDENT 1948

MYCOLOGIA

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VOL. XLI MARCH–APRIL, 1949 NO 2.

A REVISION OF THE CLASSIFICATION OF THE ASCOMYCETES WITH SPECIAL EMPHASIS ON THE PYRENOMYCETES [1]

JULIAN H. MILLER *

(WITH 37 FIGURES)

Identifications in this, the largest class of the Fungi, have always been very difficult for all except specialists in small groups. Taxonomic units have been erected on gross morphological characters which fluctuate with varying environments or ecological habitats. Important fundamental characters such as those of the ascus, or those of the ascocarp centrum, have been largely ignored in establishing relationships. This has resulted in artificial groupings and keys of little practical use especially in the identification of random collections.

Since the turn of the century there have been very few comprehensive monographs of the Ascomycetes, and so the mycologist still has to fall back on one of two systems: that of Saccardo (**22**) in the Sylloge Fungorum, or the one in Engler und Prantl (**6**), Natürlichen Pflanzenfamilien. This is in spite of a wealth of discovery of more fundamental, and therefore more constant characters than those used in the above volumes.

In this paper the writer has attempted groupings based primarily on characters of the ascus correlated with ascocarp centrum char-

[1] Lichens are not treated in this paper.

* Presidential address given at the Washington meeting, 1948.

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acters. It is believed that this will result in a more natural system as well as in keys that can be used by the student as well as the expert.

SYSTEMS OF CLASSIFICATION NOW IN USE

With the publication of the Pflanzenfamilien in 1897, a classification of the Ascomycetes was presented which has been the one generally taught in American mycology courses. This has been due chiefly to the widespread use of the two books by F. L. Stevens (**25, 26**), which are for the most part translations of the above.

From the modern point of view the orders or suborders are apparently collections of unrelated forms. Many changes in the classification have been suggested, but so far they have not been incorporated into an over all system.

The Helvellineae, Pezizineae and Phacidiineae have been revised on the character of the ascus opening. Seaver (**24**) has monographed the Operculates, and Nannfeldt (**19**) the Inoperculates in part.

In the Hysteriineae, Nannfeldt (l.c.) has taken the Hypodermataceae out of the order and placed the members in the Helotiales, and has made the Ostropaceae the type of a new order, the Ostropales. In addition he has placed the Hysteriaceae, sensu stricto, in the Pseudosphaeriales.

The Perisporiales have also been broken up, and the Microthyriaceae placed in the Hemisphaeriales by Theissen and Sydow (**28**). They include in the Perisporiales only the Erysiphaceae and Perisporiaceae. The recent viewpoint is to consider the Erysiphaceae quite distinct and to erect the order Erysiphales. The concept of *Meliola* Fr. and related genera cannot be satisfactorily fixed in the system until more species have been investigated.

The Hypocreales, separated on color and texture alone, have long been recognized as an artificial assemblage. The scolecosporous forms with the ascus cap have been made the basis of another order by Nannfeldt (l.c.), the Clavicipitales.

The other large groups represented by species of *Nectria* Fr., *Hypocrea* Fr., and *Hypomyces* (Fr.) Tul. and their apparent relatives, have not been studied sufficiently from the standpoint of their

developmental histories to warrant either splitting them into families or placing them with any other Pyrenomycetes.

The Dothideales and Sphaeriales are also artificial groupings. Species in which the locules lack separate walls and in which more than one occurs in a stroma were placed in the former. If perithecia with walls are produced, the species were placed in the latter. If only one locule is present in a stroma, by interpretation the species was placed in the Sphaeriales because this could not be distinguished from a single perithecium with a wall.

Von Höhnel (**9**, **10**) first focussed attention on the centrum or "nucleus" characters, and established the pseudosphaeriacous centrum as opposed to the sphaeriacous centrum. The former was distinguished by each ascus being in a separate locule formed of interthecial stromal threads. In the Sphaeriales one finds free paraphyses and no interthecial threads. This question has been discussed at length by von Höhnel (l.c.) Theissen and Sydow (**29**), Petrak (**21**), Nannfeldt (**19**), and the writer (**16**, **17**, **18**).

In Saccardo's Sylloge Fungorum the limits of the families correspond in large part to those of the orders or suborders, or in some cases to the families, of the Engler und Prantl system. The chief differences lie in the primary separations being based on spore characters in the former as opposed to position of the ascocarp relative to the substrate, amount of stroma, and other such artificial characters in the latter. Of the two the Saccardo system seems to bring together more closely related forms than the other system. However, the major groupings are open to the same criticism in both.

PRESENT REVISION

The writer proposes suggestions for a revision of the fungi previously placed in the Sphaeriales, the Dothideales, and the Hypocreales, and demonstrates the changes in a key at the end of this paper. His formerly expressed ideas as well as some of those of von Höhnel, Theissen and Sydow, Nannfeldt and others are incorporated in these changes. In addition there are a few suggestions for rearrangements in other orders. However, only separations of orders and families are attempted, as it would now be

impossible to establish a complete revision, especially down to species. This awaits the work of specialists in the groups.

Criteria of relationship are based on characters of the ontogeny, as well as of the mature apothecial or perithecial centrum, and on morphological characters of the ascus and ascospores. Such characters as position of the ascocarp relative to the substrate, or color or extent of stroma, are not considered fundamental and are ignored in the keys.

Primitiveness is thought to consist of globose to oblong or widely clavate asci, usually with a uniformly thickened wall, and opening by splitting or gelatinization of the outer wall and elongation of the inner, which in turn dissolves, freeing the spores. Also, the asci are variously scattered on mycelium or rather irregularly grouped in an ascocarp. Lastly, the asci arise following a union of an archicarp with an antheridial hypha.

The high point in development would be exhibited by cylindrical asci closely compacted in an ascocarp, usually with thin lateral walls, much thickened at the apex, and opening by a pore or lid. The asci here arise from a much branched ascogenous hyphal system, following the union of free spermatia with the trichogyne of an archicarp. The idea that spermatia and archicarps are present, and then later ascogenous hyphae arise apogamously from basal vegetative cells, could well have resulted from imperfect cytological technique. In many cases it is practically impossible to trace the connections between the asci and the archicarp with the usual paraffin section method, but that doesn't justify the conclusion of apogamy.

The writer has recognized these types of asci and ascocarp centra.

1. The globose or broadly clavate asci without a pore lie at irregular levels throughout loose or pseudoparenchymatous tissue composed of ascogenous hyphae. As evolution proceeds the asci are brought into one locule and each ascus grows up into the stroma. The monascal locule partitions are then the remnant stromal elements. These tendencies are exhibited in the Plectomycetes.
2. The clavate asci with no pore arise in a basal fascicle and in growing upward dissolve out a stromal locule. These are

multi-ascal locules, with no interthecial threads. This is the central tendency of the Dothideales.

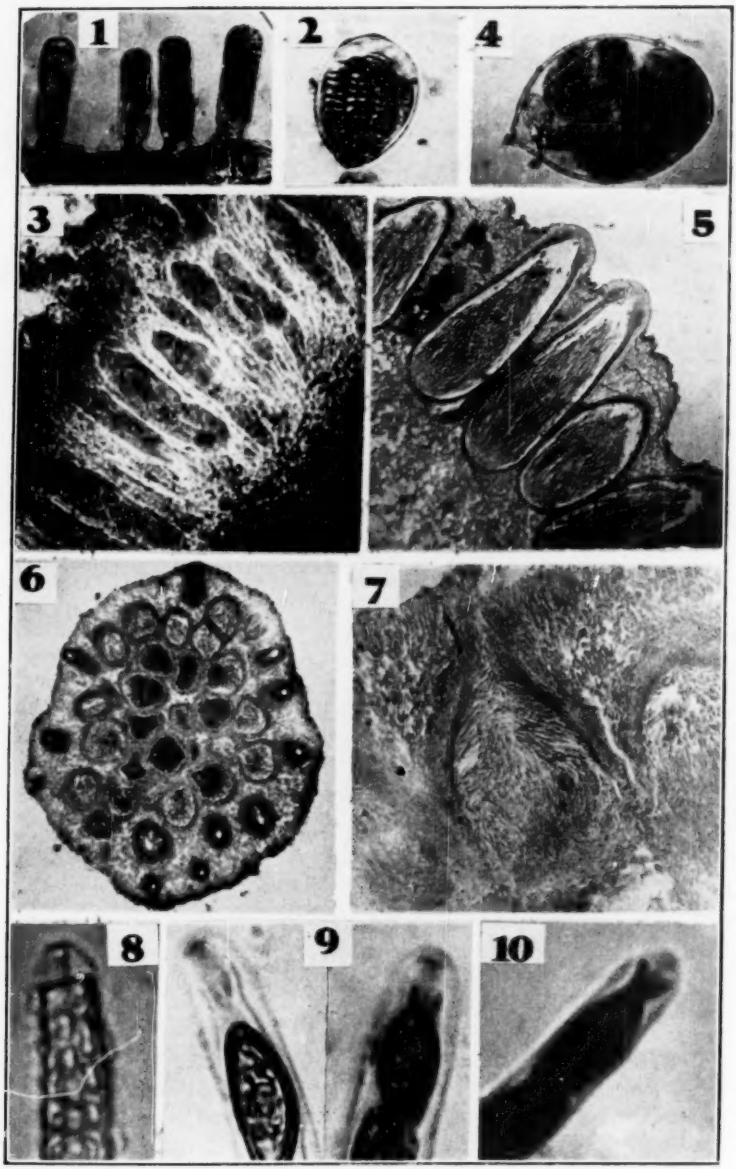
3. The clavate to cylindrical asci with no pore arise from an entire wall-layer in a stroma and grow upward between filiform threads, which are connected at the top and bottom of the locule. Following the presence of the archicarp there is a fan-shaped array of deeply staining threads that grow downward toward the base of the locule, ultimately appearing as a palisade of vertical threads. Then the asci arise from a position at their bases and grow up between them. These threads are present before the asci arise and are here designated as pseudoparaphyses. They are characteristic of the Pseudosphaeriales, Microthyriales, Hysteriales, and Lophiostomatales.
4. The clavate to cylindrical asci with a pore arise between apically free threads, which usually precede them in development. This is the writer's concept of paraphyses. The ascocarp wall is a special structure developed from the basal cells of the archicarp and is not part of the stroma. This type of centrum is characteristic of the Sphaeriales and the Discomycetes.

3. This type has been discovered by Luttrell (**15**) in *Sphaerostilbe aurantiicola* of the Hypocreales. There is a true wall to the perithecium and periphyses in the ostiole, but vertical threads or pseudoparaphyses precede the asci as in the Pseudosphaeriales. The asci are as in type 4. This is a combination of types 3 and 4 in part. Should this prove characteristic of many of the species of the Hypocreales it would provide a 5th trend in Ascomycete development.

HEMIASCOMYCETES

Endomycetales and Exoascales

These follow the usual pattern and apparently represent primitiveness as well as simplicity. Nannfeldt (l.c.) leaves the Exoascales out of his key because of uncertain position, and places the Endomycetales coordinate with the Hemiasci (Dipodascaceae). The writer uses the same ordinal limits as Wolf and Wolf (**32**). *Taphrina caerulescens* (Mont. & Desm.) Tul. shows the characters of the ascus (FIG. 1).



FIGS. 1–10. Types of spores, asci and ascocarps in the Ascomycetes.

EUASCOMYCETES

The old concept of asci in ascocarps is retained. Nannfeldt (l.c.) divided this subclass into 1. Plectascales, 2. Ascoloculares, and 3. Ascohymeniales. His Plectascales contain most of the same groups as are found in the Plectascineae of the Pflanzenfamilien or the Plectascales of Gwynne-Vaughan and Barnes (**8**) or Bessey (**1**) (sub Aspergillales), or Wolf and Wolf (l.c.) (sub Eurotiales). His Ascoloculares and Ascohymeniales, however, are new groupings. The former comprises all of the Pyrenomycetes with asci in locules, including even the Myriangiales and Hysteriales. The Ascohymeniales on the other hand include forms with a hymenium of asci and apically free paraphyses.

The writer thinks it preferable to keep the old separations of Plectomycetes, Pyrenomycetes and Discomycetes with some modifications. For example, in Nannfeldt's Ascoloculares there are at least three ontogenetically different ascal locules, or the writer's types 1, 2 and 3. In the Myriangiales the pseudoparenchymatous locule tissue is composed of ascogenous hyphae and the asci tend to be globose which places that order in the Plectomycetes. Then in Nannfeldt's Pseudosphaeriales he has the *Pleospora*-type with connected threads between the asci, and the *Dothidea*-type with no threads. Neither von Höhnel nor Theissen and Sydow distinguished between the monascus locule composed of the vertical threads that are in the centrum before the asci appear, and the remnants of stromal elements due to the asci pushing up in the stroma as in the higher Myriangiales, such as in *Dothiora subtropica* (Wint.) Mill. & Burt. (FIG. 3).

It appears more logical to retain the term Discomycete for the Ascohymenial forms with exposed hymenium and Pyrenomycete for those with inclosed asci. Then all of Nannfeldt's forms in his Ascohymeniales with true perithecia should go in either the Sphaeriales or Hypocreales. In his Ascoloculares forms with the *Dothidea-Mycosphaerella* type of development should be placed in the Dothideales, and those with the pseudosphaeriacous type in the Pseudosphaeriales, Lophiostomatales, Hysteriales, or Microthyriales.

PLECTOMYCETES

Eurotiales, Myriangiales and Erysiphales

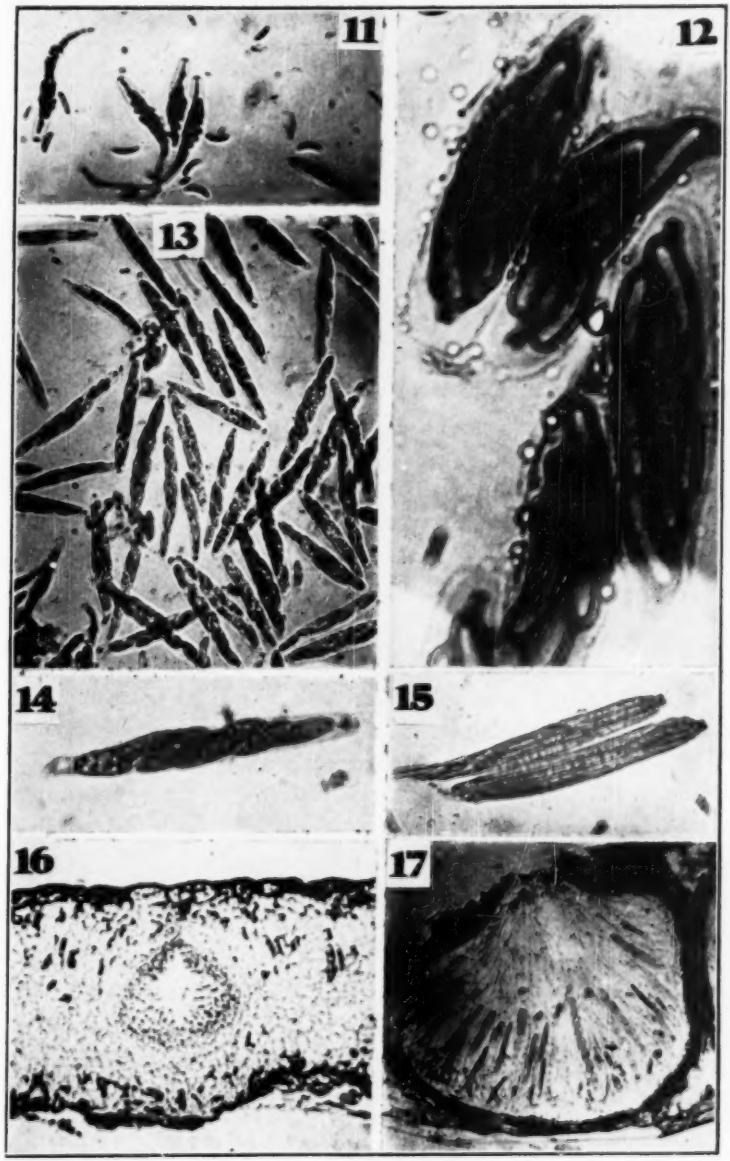
These are connected by the globose to semiglobose asci generally with uniformly thickened walls opening by the outer wall splitting, and ascocarps with no paraphyses or other threads and with no definite manner of opening (FIG. 2. *Myriangium Duriaei* Mont. & Berk. and FIG. 4. *Microsphaera Alni* DC. ex Wint.). The irregular arrangement of the asci, surrounded by ascogenous hyphae, and the liberation of spores within the ascocarp are characteristic except in the Erysiphales. In the latter the asci are typical, but in basal tufts.

Nannfeldt (l.c.) added the Ophiostomataceae (Ceratostomataceae) and Chaetomiaceae to his Plectascales because of asci with walls that deliquesce very early and liberate masses of spores within the ascocarp. There are no interthecial threads. This seems logical, but both groups are exceptions in possessing definite ostioles. The asci of the Plectomycetes are usually formed on the ends of hyphae or in chains, and ascus hooks are very rarely present.

The name Perisporiales is dropped because the order as delimited by Lindau (**6**) in the Pflanzenfamilien comprises forms that are now known to belong in several other orders. The most definite and clear-cut group with well-known life histories is the Erysiphaceae, so the name is changed to the Erysiphales as is done by Gwynne-Vaughan and Barnes (**8**). Not enough is known of the developmental characters of the Meliolaceae to indicate their affinities. According to Graff (**7**) there is an outer stroma beneath which the perithecium is hidden, with paraphyses and periphyses in an ostiole; however there is an oogonial and antheridial stalk. The sex organs are similar to those of the Erysiphaceae, but the centrum characters are apparently those of the Sphaeriales.

DISCOMYCETES

This subclass includes species with a hymenium composed of asci with a pore or lid and apically free paraphyses, exposed at least at maturity. This comprises the Discomycete part of Nann-



FIGS. 11–17. Types of spores, asci and ascocarps in the Ascomycetes.

feldt's Ascohymeniales. This is also the old concept except for the Hysteriales in part, which are placed in the Pyrenomycetes in this revision. The Hypodermataceae are transferred to the order Helotiales as is done by Nannfeldt.

INOPERCULATES—*Ostropales and Helotiales*

These orders follow Nannfeldt's concept, and the difference between the ascus cap and threadlike canal in the former, and the wide pore of the latter can be easily distinguished. The characters of ascus and spores in the Ostropales are very similar to those of the Clavicipitaceae.

OPERCULATES—*Pezizales and Tuberales*

The Pezizales here include the Pezizaceae and the Helvellaceae (Elvelaceae) as limited by Seaver (**24**). The Tuberales are placed at the end of the Discomycetes with the idea that they have evolved from the Pezizales. This is the usual position. It is very difficult to place them in a key with characters that will separate them from the hypogeic Plectomycetes.

PYRENOMYCETES

The writer includes in this subclass all fungi in which the asci are borne in parallel series in a closed fruiting body which ultimately opens by a definite pore or slit. The exception would be the Hypodermataceae in the Helotiales.

SPHAERIALES

This order is revised to include only forms with the writer's (**16**) previous concept of a true perithecium; that is, walls arising from an archicarp, soon forming a globose hollow ball with a hymenium of asci and apically free paraphyses developing from a wall layer, and an ostiole formed by extension of the wall tissue and lined with periphyses. The mature asci have thin lateral and thick apical walls penetrated by a pore. The spores are typically liberated through the pore.

Young and mature perithecia are illustrated by Brown's figures (**2**: FIGS. 24–31) of *Xylaria*, and by the writer's figures 16, of

Phyllachora Lespedezae (Schw.) Sacc., 5, of *Cordyceps militaris* (L. ex Fr.) Lk., and 17, of *Phyllachora graminis* (Pers. ex Fr.) Fckl.

In making identifications, if there is more than one perithecium in the stroma, the presence or absence of a perithecial wall is sufficient. The difficulty usually comes in separating forms with single perithecia from uniloculate stromata, especially if one does not have access to early stages. The character of the ascus should separate these. If the ascus wall is apically thickened and possesses a pore, the fungus belongs in the Sphaeriales. If a pore is lacking and the outer wall splits when the spores are ejected, the fungus is a loculate form. An additional separation is that in the Sphaeriales the ascus gives a blue reaction in iodine, a character not present in the other orders.

CLAVICIPITACEAE

This family, with *Claviceps* Tul. as the type, constitutes a well defined group. There is the very marked cap at the apex of the long cylindric ascus (FIG. 8), and filiform spores that usually break up into segments at maturity. The pore is very narrow and penetrates the cap. The ascospores in ejection apparently do not pass through the pore, but instead the entire cap is pushed off and the spores extruded. The walls of the perithecia are pseudoprosenchymatous, and are sharply defined in all species studied. The asci arise from a basal plectenchyma and never lie in a wall layer as in members of the other families. Paraphyses and periphyses are formed, but deliquesce early in most species.

Species of the genera *Claviceps, Cordyceps* (Fr.) Lk., *Balansia* Speg., *Dothichloë* Atk., *Ascopolyporus* Moll., and *Epichloë* Tul. have been studied, and in all cases the perithecia lie in a stroma which is either stalked or sessile. All are parasites on grasses or on insects.

Nannfeldt (l.c.) makes this group an order, and separates it from the rest of his Ascohymeniales by the lack of a special perithecial wall and paraphyses. This is at variance with findings of the writer. Figures 6–7, *Claviceps purpurea* (Fr.) Tul. and figure 5, *Cordyceps militaris* (L. ex Fr.) Lk. all show distinct walls.

In addition paraphyses are found in young perithecia. Jenkins (**12**) with *Cordyceps capitata* (Holmsk. ex Fr.) Lk. (*Cordyceps agariciformia* (Bolt.) Seaver) finds a true wall and ostiole and paraphyses-like threads and periphyses.

Luttrell (**15**), in reviewing the literature of the Hypocreales, mentions five types of development. His fourth type consisting of poorly developed wall and no paraphyses, and including the *Cordyceps-Claviceps* group, is hardly correct as there are walls and paraphyses, or "paraphyses-like threads" as Jenkins calls them, at an early stage. His fifth type, consisting of asci formed in locules in a stroma with perithecial wall, ostiole and paraphyses lacking—*Epichloë typhina* (Pers. ex Fr.) Tul., is also incorrect according to the writer's sections.

XYLARIACEAE

This is one of the oldest and best recognized groups of Ascomycetes. It consists of many genera and species widely scattered throughout the world. The entire series constitutes an excellent phylogenetic tree with few missing links. The members are peculiarly susceptible to changes in color, size, and shape of the stroma under variable environments, so the number of so-called species in the literature has been tremendous.

The perithecia have well defined pseudoprosenchymatous walls, and are either single or many in a stroma. The asci are cylindric, stalked, persistent, formed within a wall layer, possess a most peculiar crown at the base of the ascus pore, which ends in a wide plug (see FIG. 9, *Hypoxylon tinctor* (Berk.) Cke. and FIG. 10, *Xylaria Hypoxylon* (L. ex Fr.) Grev.). Spores are ejected through the pore into the perithecial neck and accumulate in black masses just outside of the ostiole. These spores are uniseriate, inequilaterally elliptical, 1-celled, dark colored, and have a longitudinal germ pore. Paraphyses are copious, filiform, branched, and persistent until late in the life of the perithecium, when they gelatinize, especially up near the ostiole.

Small 1-celled conidia, or probably in some cases spermatia, are borne on the surface of the stroma or in cavities.

The type of the *Xylariaceae* should be the genus *Xylaria* Hill. ex Grev. and the type species, *X. Hypoxylon*.

Nannfeldt (l.c.) designated this the most typical family for his Sphaeriales. It should be the type family as it exemplifies the concept most fully.

Genera that have been placed in other families by Lindau (**6**) such as *Anthostoma* Nits., *Anthostomella* Sacc., *Sordaria* Ces. & De Not., and *Rosellinia* De Not., have the special ascus crown character and so should go in the Xylariaceae.

The exceptional genus is *Clypeosphaeria* Fckl. In *C. mamillana* (Fr.) Lamb. all characters are well within the family concept, even to the possession of the ascus crown, but the spores are three-septate. Nannfeldt (l.c.) also noted the relationship to the Xylariaceae after studying *C. Notarisii* Fckl., the type.

Lindau (**6**) placed the genus *Xylobotryum* Pat., with two-celled ascospores, in this family. The writer has the type species, *X. andinum* Pat., and it has asci not thickened at the apex and neither crown nor pore, and has two-celled ascospores, which should exclude it.

ALLANTOSPHAERIACEAE

This family was created by von Höhnel (**11**) for all typical Pyrenomycetes with allantoid ascospores. He divided the family into four subgroups. One, the Diatrypeen v. Höhn., possesses asci located in a wall layer and which have a refractive ring. It belongs, according to Nannfeldt (l.c.), in the Sphaeriales. Further, he has included the genera *Cryptosphaeria* Grev., *Cryptovalsa* (Ces. & De Not.) Fckl., *Diatrype* Fr., *Diatrypella* (Ces. & De Not.) Sacc., *Eutypa* Tul., *Eutypella* (Nits.) Sacc., and *Quaternaria* Tul. Nannfeldt (l.c.) sets this group up as a family, the Diatrypaceae, and places it in the Sphaeriales. Wehmeyer (**30**) and also Wolf and Wolf (**32**) use the name Allantosphaeriaceae.

The writer thinks there will be less confusion if von Höhnel's name is retained and the group characterized as follows:

Perithecia pustulate or dispersed, usually sunken in bark or wood, with elongate necks, ostioles usually sulcate. Asci in a wall layer, clavate, with attenuate stalks, with thick apical walls, with refractive ring in pore, persistent; with ascospores chiefly 1-celled, allantoid, and light colored. Paraphyses filiform, branched, copi-

ous and persistent. Figure 11, *Eutypella fraxinicola* (Cke. & Pk.) Sacc., shows the characters of the ascus.

Conidia usually 1-celled, hyaline, borne on surface or in locules of stroma.

The type of the family should be *Diatrype* Fr., and of the genus *D. disciformis* Hoffm. ex Fr. Representative genera should be *Diatrype*, *Diatrypella*, *Eutypa*, and *Eutypella*. This is the concept of Wehmeyer (l.c.) for the family, except for the genus *Anthostoma*, which the writer places in the Xylariaceae because of the crown in the ascus.

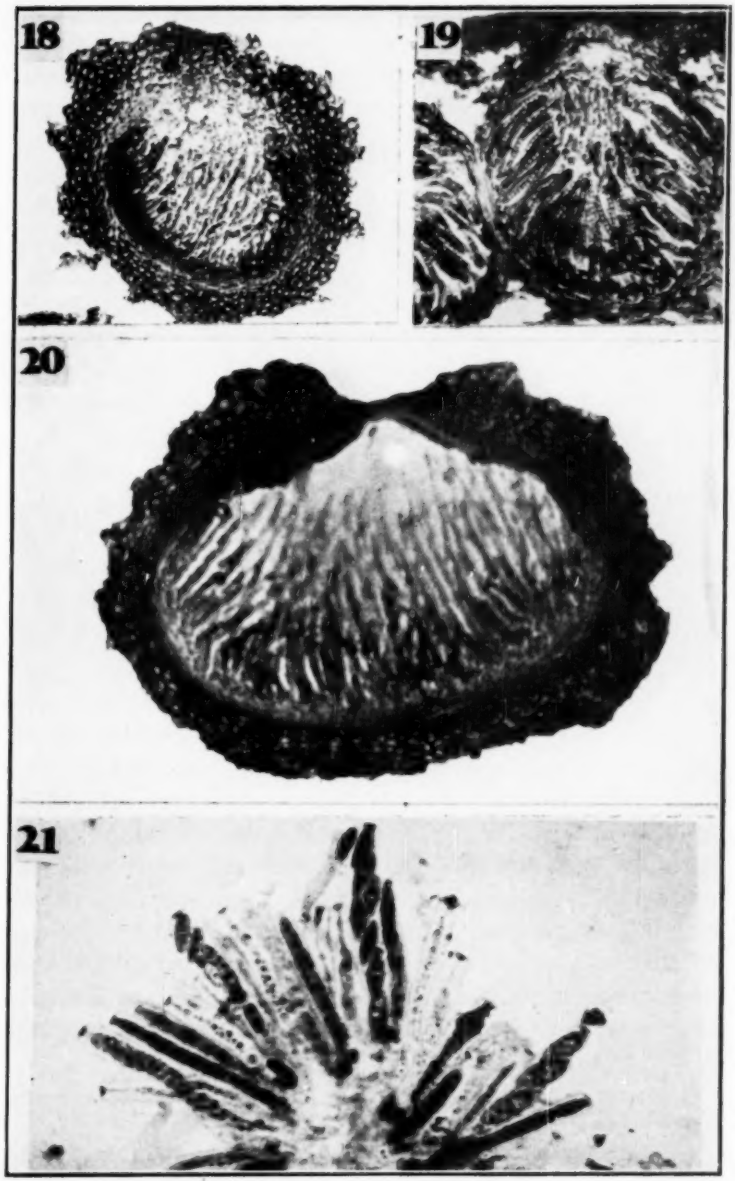
DIAPORTHACEAE

This group was first delimited by von Höhnel (**10**) to comprise forms with his "diaportheen nucleus." Nannfeldt (l.c.) erected an order, the Diaporthales, coordinate with the Sphaeriales. Further, he placed the forms with allantoid spores but with the diaportheen centrum in another order, the Valsales. The writer retains essentially the von Höhnel grouping, or the more recent concept of Wehmeyer (**30**), without limiting it to forms on wood or bark.

The characters of the family consist of perithecia usually with long necks, one or more in a stroma or pseudostroma, or in no recognizable stroma, with rounded ostioles, not sulcate. Asci are in a wall layer, clavate, short-stalked, thin-walled except at the apex and that is very thick, penetrated by a pore containing a light-refractive ring, at maturity free from base and becoming loose in the perithecial centrum, completely filling it. In most cases spore ejection is through the pore, but sometimes the wall of the ascus dissolves and the spores collect in a gelatinous mass at the apex. Paraphyses are at an early stage seen as broad, free pointed, bands interspersed with some narrow filiform ones. The entire mass gelatinizes at maturity.

Conidia are usually borne in exposed layers, in acervuli, or locules in a stroma. They vary in shape and color as much as the ascospores.

The genus *Diaporthe* Nits. should be the family type and *D. eres* Nits. the type of the genus. The genera placed here include ones



FIGS. 18–21. Types of spores, asci and ascocarps in the Ascomycetes.

in both the Diaporthales and Valsales of Nannfeldt (l.c.), and in part ones in the families Clypeosphaeriaceae, Gnomoniaceae, Melogrammataceae, Melanconidaceae, and Valsaceae of Lindau (**6**). The chief genera are *Cryptospora* Tul., *Cryptosporella* Sacc., *Endothia* Fr., *Diaporthe* Nits., *Gnomonia* Ces. & De Not., *Gnomoniella* Sacc., *Hercospora* Tul., *Linospora* Fckl., *Mamiania* Ces. & De Not., *Massariovalsa* Sacc., *Melanconis* Tul., *Ophiodothella* v. Höhn., *Pseudovalsa* Ces. & De Not., *Valsa* Fr., and *Valsaria* Ces. & De Not.

Some of the above groups vary in at least one character. In *Linospora populina* Pers. ex Schrt., *Ophiodothella Ingae* (P. Henn.) Th. & Syd. and *Valsaria rubricosa* (Fr.) Sacc. the apical ring is very distinct, but the asci do not float out in water as easily as in *Diaporthe* species. Then in *Massariovalsa sudans* (Berk. & Curt.) Sacc. and *Pseudovalsa sigmoidea* (Cke. & Ell.) Sacc. the refractive ring is not plain.

The character of the asci floating out in water is shown in figure 13, *Diaporthe oncostoma* (Duby) Fckl., and the sessile ascus with the light refractive ring is illustrated in figure 14, *D. oncostoma,* figure 12, *Valsa ambiens* Pers. ex Fr., and in figure 15, *Cryptospora cinctula* (Cke. & Pk.) Sacc.

PHYLLACHORACEAE

This family has historically been placed in the Dothideales by Lindau (**6**), Stevens (**25**), and Theissen and Sydow (**27**). The type of the family is *Phyllachora* Nits., and of the genus *Ph. graminis* (Pers. ex Fr.) Fckl. The latter was supposed to have contained no true perithecia, but instead locules in a flat stroma sunken in the leaf (see Stevens [**25**], *fig.* 157). This concept was refuted by Orton (**20**), who showed that each perithecium contained a special wall, and that the blackened hyphae which form the clypeus arise in the epidermis or cuticle of the leaf as lateral proliferations of the ostiolar tissue.

There are a great number of specific names in the literature and all of them have been based on fungi found on living leaves of many plants. However, only the ones on grasses have been studied in detail and they conform to the type. Of the others, the

writer has studied only *Ph. Lespedezae* (Schw.) Sacc. (FIG. 16), and it has a true perithecium but in a stroma.

The characters of the family as delimited here follow:

Perithecia globose, with thin walls, lying in leaf tissue or in a stroma and maturing in living leaves, with plane ostioles and sometimes with a clypeus formed by the lateral proliferation of ostiolar hyphae. Asci persistent in a wall layer, cylindric, very short stalked, with walls fairly uniform in thickness, not especially thickened at apex, with wide pore in center of apex, blue with iodine. Ascospores uniseriate. Paraphyses copious, persistent, filiform, and branched.

This group contains at present only the genus *Phyllachora*. Whether other genera should be included depends on a thorough study of forms placed in the family by Theissen and Sydow (**27**) and others. Their separation of the old genus into several based on position of the fructification in the leaf does not seem to have merit.

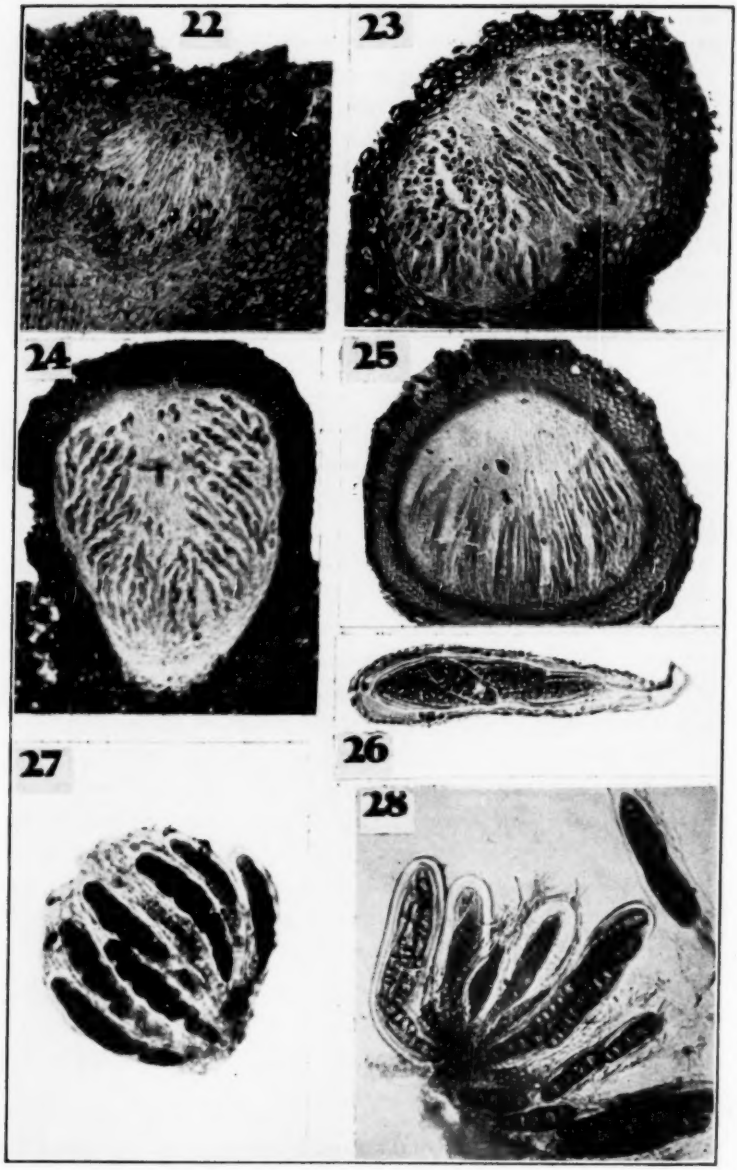
Nannfeldt (l.c.) places *Phyllachora* in a family, the Polystigmataceae, which is based on *Polystigma rubrum* (Pers. ex Fr.) Chev. This forms a solid fleshy stroma with sunken perithecia in the leaf. The perithecia mature only after overwintering in the dead leaf. Whether this form should be placed in the same family with *Phyllachora* would depend on the results of more study.

Phyllachora species on grasses in the genera *Andropogon* L., *Elymus* L., *Panicum* L. and *Triodia* R. Br. have been studied by the writer, and they possess a true wall. At an early stage they show free paraphyses and asci growing into an inclosed hollow. Figure 17, *Phyllachora graminis*, shows these characters. Figure 16, *Ph. Lespedezae*, shows a very young perithecium in a solid fungous stroma, with typical characters of the Sphaeriales.

HYPOCREALES

This order is tentatively delimited here as set up by Lindau (**6**) with the exception of the Clavicipitaceae, which is placed in the Sphaeriales.

It has been thought that all of the forms were characterized by possession of a perithecial wall. For example, Clements and Shear



FIGS. 22–28. Types of spores, asci and ascocarps in the Ascomycetes.

(**4**) say that the Hypocreales are distinguished from the Dothideales by the presence of a distinct perithecial wall and fleshy bright colored perithecia. They make the group a family and place it in the Sphaeriales. The writer (**18**) has done the same thing in the past. However, in view of recent knowledge this may have been a mistake, except for the Clavicipitaceae. There are other large groups such as species of *Nectria* Fr., *Hypomyces* (Fr.) Tul., *Hypocrea* Fr., and others whose life histories have not been studied sufficiently to accurately judge their relationships. It is possible that there are other characters that would prevent this alignment in the Sphaeriales. For example, Luttrell (**15**) has made a very detailed study of *Sphaerostilbe aurantiicola* (Berk. & Br.) Petch and he finds that there is a true perithecial wall and a periphysate ostiole, but no paraphyses, and there are vertical threads or pseudoparaphyses attached in the roof. Here, then, are wall and ostiole characters of the Sphaeriales combined with centrum characters of the Pseudosphaeriales. Luttrell also finds the vertical hyphae in *Nectria cinnabarina* Tode ex Fr. and records similar conditions in *Nectria Ribis* and *Hypocrea gelatinosa* (*Creopus gelatinosus* (Tode ex Fr.) Lk.). The writer finds this centrum in *Nectria cinnabarina*, *Creopus gelatinosus*, *Nectria ochroleuca* (Schw.) Curt., *Nectria coccinea* Pers. ex Fr., *Nectria galligena* Bres., *Thyronectria austro-americana* (Speg.) Seeler, *Hypocrea lenta* (Tode ex Fr.) Berk. and Br. and *Hypomyces lactifluorum* (Schw. ex Fr.) Tul.

The presence of the vertical threads has evidently been overlooked in *Hypomyces*, *Hypocrea*, and *Podostroma* Sacc. Both Lindau (**6**) and Clements and Shear (**4**) state paraphyses lacking for these genera. In other cases these threads have been called paraphyses.

Figures 18 and 20 show the vertical threads in *N. coccinea* and figure 19 in *Hypocrea lenta*. Figure 21 shows the pores in the asci of *Nectria galligena*.

From the present insufficient evidence, then, it seems logical to leave the Hypocreales, with the exception of the Clavicipitaceae, in the status quo until more work has been done. Then if most of them do have the above characters we will have an order charac-

terized by the presence of a definite perithecial wall, but with the centrum of the Pseudosphaeriales. At present all forms with free paraphyses should be removed to the Sphaeriales, and those with asci in pseudoparenchyma to the Dothideales, and those with the vertical threads but no wall to the Pseudosphaeriales.

DOTHIDEALES

In revising this group no change is necessary in the original concept of the type. The name came originally from *Dothidea* Fr., and *Dothidea Sambuci* Fr. is the type of the genus. This species is not the first one described, but it is the element with which the name has been permanently associated, and it fixes the generic name, and also the family and ordinal names as commonly applied. Theissen and Sydow (**27**) did not have sufficient basis to change this name to *Systremma* Th. & Syd.

The present idea, then, is in reality the very old one of ascus locules in a stroma with no special wall, and each locule, as in *Dothidea Sambuci,* contains a persistent fascicle of asci with no interthecial threads. The locule opens by a lycigenously formed pore in the stromal wall. This concept is emended to include uniloculate forms with this centrum. The ascus is uniformly thickened and opens as in the Pseudosphaeriales by the outer wall splitting and the inner protuding. (See Wolf and Wolf **32**: *fig.* 80.)

Theissen and Sydow (**29**) place the uniloculate stromata such as in *Mycosphaerella* Johans. in the Pseudosphaeriales, but as pointed out previously the interthecial tissue is ontogenetically different in the two concepts.

In the group as monographed by Theissen and Sydow (**27**) the Phyllachoraceae go in the Sphaeriales and all forms with pseudoparaphyses, such as *Auerswaldiella puccinioides* (Speg.) Th. & Syd., must be transferred to the Pseudosphaeriales as emended by the writer.

At present one could arbitrarily separate the group into two families as follows: multiloculate stromata, Dothideaceae; uniloculate stromata, Mycosphaerellaceae. This will not always maintain a sharp separation because there are species of *Mycosphaerella*

such as *M. effigurata* (Schw.) House in which the stroma often contains more than one locule. This is also true of *Guignardia Bidwellii* (Ell.) Viala & Ravaz.

The multiloculate stroma is shown in figure 33, *Dothidea collecta* (Schw.) Ell. & Ev., and figure 32, *Auerswaldia examinans* (Mont. & Berk.) Sacc. Figure 34, *Dothidea collecta,* shows the ascus walls with no pore and the basal attachment producing the fascicle. Figures 35–36, *Mycosphaerella effigurata,* show the characteristic centrum and ascus.

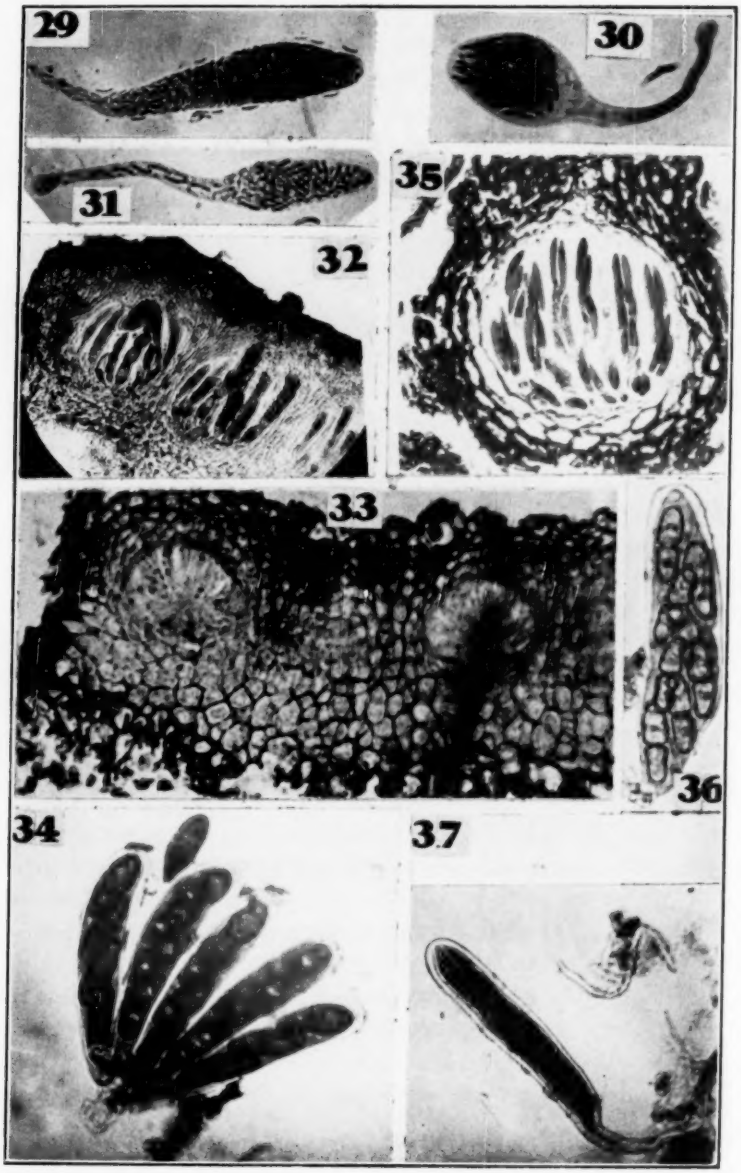
PSEUDOSPHAERIALES

This order is based in part on the Pseudosphaeriaceae concept of von Höhnel (**9**), Theissen and Sydow (**29**), Petrak (**21**) and others. The writer (**16**) discussed this question in detail some time ago, and there is the more recent discussion by Nannfeldt (**19**).

Previously the writer has thought that the threads (see FIG. 22) in the centrum attached at top and bottom were remnants of stromal tissue in process of dissolution by developing asci. Since then sections of young stages of many species have shown the presence of these threads before the asci even begin to form. The archicarp first develops in the stroma, followed by a fan-like downward growth of threads from the position of the archicarp. Then as the stroma develops the threads elongate and asci arise at their bases. The connection between these vertical threads and the asci has never been satisfactorily explained. Sartoris and Kauffman (**23**) and Cavara and Mollica (**3**) thought the young asci were formed in the threads. Dodge (**5**) with a *Leptosphaeria* and Wehmeyer (**31**) with a *Pseudotrichia* thought there was no connection with the asci.

This stage in the ontogeny has not been found in the group delimited here as the Sphaeriales. Other characters that set the Pseudosphaeriales apart are the thick ascus wall not penetrated by a pore, and the method of splitting of the outer wall for spore ejection.

This order, then, is characterized by the asci being borne in locules, in a wall layer or between pseudoparaphyses, and by one



FIGS. 29–37. Types of spores, asci and ascocarps in the Ascomycetes.

to many locules in a stroma opening by a lysigenously formed pore. The thickness of the locule wall is no criterion of the stromal character. The wall cells tend to be pseudoparenchymatous. The other characters are the connected threads and the uniformly thickened ascus wall with no pore.

Genera placed here are ones previously found chiefly in Lindau's (**6**) families, Sphaeriaceae, Amphisphaeriaceae, Pleosporaceae, and Cucurbitariaceae, and in the Dothideales of Theissen and Sydow (**27**). Species studied by the writer and found to have this centrum have been enumerated in the Plant Disease Reporter (**18**).

At present there seem to be two tendencies that might well represent families:

Ascus walls thick, pseudoparaphyses not gelatinizing at maturity
Pleosporaceae

Ascus walls uniformly thin, pseudoparaphyses gelatinizing at maturity, forming a jelly-like mass in the roof of the stroma
Nitschkiaceae

Mature locules are shown in figure 23, *Auerswaldiella puccinioides*, figure 24, *Dibotryon morbosum* (Schw. ex Fr.) Th. & Syd. and figure 25, *Lasiosphaeria ovina* (Pers. ex Fr.) Ces. & De Not. All of them show the attached threads. The ascus characters of thick wall and no pore are shown in figure 26, *Botryosphaeria Ribis* (Tode ex Fr.) Gr. & Dug., figure 27, *Pleospora aurea* Ell., and figure 28, *Massaria Argus* (Berk. & Br.) Fres.

The forms placed in the Nitschkiaceae, figure 29, *Fracchiaea heterogena* Sacc., figure 30, *Coronophora oötheca* (Berk. & Curt.) Sacc., and figure 31, *Cryptosphaerella annexa* (Nits.) v. Höhn., have allantoid spores and the asci show no pore. The latter constitutes an easily recognizable character distinguishing them from the Allantosphaeriaceae or Diaporthaceae.

MICROTHYRIALES

The writer prefers this name to that of Hemisphaeriales because the Microthyriaceae with the shield-shaped ascocarp of radiating structure should be the type group. Only a few of the species have been investigated morphologically; however Nannfeldt (l.c.) placed

them in his Ascoloculares with the monascus locule. Luttrell (**14**) with *Myiocopron Smilacis* (De Not.) Sacc. found the ascocarp to be an ascostroma containing a single locule filled with asci and pseudoparaphyses. Then in *Morenoella quercina* (Ell. & Mart.) Th., Luttrell (**13**) found that the ascocarp is also a stroma and that each ascus originates individually and by its expansion creates a locule within the stroma. He suggests that possibly all members of the order described as "aparaphysate" are of this type.

The order Microthyriales, then, should be limited to species with dimitate stromata bearing locules, one or more, with asci between pseudoparaphyses. In this manner they would be closely related to the Pseudosphaeriales.

Forms now in this order with shield shaped ascocarps, in which the asci form single locules by dissolving stromal elements, and which have no pseudoparaphyses, should be connected with the higher Myriangiales such as the Dothioraceae.

LOPHIOSTOMATALES

The writer has made no detailed study of any members of this order, but places them in a separate order rather than as a family in the Sphaeriales (see Lindau, **6**), because of the presence of pseudoparaphyses and asci with no pore. This order then should comprise uniloculate perithecial-like stromata having connected pseudoparaphyses and opening by a slitlike ostiole. These fungi differ from the Pseudosphaeriales only in the opening.

Nannfeldt (l.c.) has these in his Ascoloculare group because of the threads in the centrum.

HYSTERIALES

The type of the order is the family Hysteriaceae obtained from *Hysterium* Tode ex Fr., which has *H. pulicare* Pers. ex Fr. for the generic type. The species here have boat-shaped ascocarps, opening by an elongate slit. The asci (FIG. 37, *Hysterographium flexuosum* (Schw. ex Fr.) Rehm) are of the Pseudosphaeriales type with uniformly thickened wall and no pore, and lie between pseudoparaphyses.

Nannfeldt (l.c.) placed the family Hysteriaceae in his Pseudosphaeriales order in the Ascoloculares. This is after he had taken out the Hypodermataceae and transferred them to the Ascohymeniales.

The Hysteriales could be derived from the Pseudosphaeriales through the Lophiostomatales. There are forms, such as those of *Bulliardella* Sacc., which are intermediate in ascocarp characters.

TENTATIVE KEY TO THE ASCOMYCETES

1. Asci not in a specialized fruiting body, but borne singly or clustered on the mycelium ..... HEMIASCOMYCETES
   2. Mycelium usually poorly developed, growth by budding common.
      3. Saprophytic; asci formed directly by fusion of cells. ..*Endomycetales*
      3. Parasitic on plants; usually causing hypertrophy; asci arising from chlamydospores ..... *Exoascales*
1. Asci borne in ascocarps ..... EUASCOMYCETES
   4. Ascocarps closed.
      5. Opening only by weathering; *asci globose* to *broadly clavate*, with walls uniformly thickened, with *no pores*, borne in tufts or irregularly arranged ..... PLECTOMYCETES
         6. Asci borne singly in locules composed of ascogenous hyphae when they are at irregular levels.
            7. Stroma lacking ..... *Eurotiales*
            7. Stroma present ..... *Myriangiales*
         6. Asci arising singly or in tufts from a basal plectenchyma *Erysiphales*
      5. Ascocarps opening by a definite small pore or slit; *asci clavate* to *cylindric* ..... PYRENOMYCETES
         8. Perithecia stalked, borne on a receptacle; neither paraphyses nor periphyses; minute parasites on insects. *Laboulbeniales*
         8. Perithecia not stalked.
            9. Asci with *apical pore*, in *perithecia*; perithecia one to many in a stroma, with a differentiated wall, with ostiole formed by the extension of the wall and lined with periphyses
               10. Asci within a wall layer of *apically free* paraphyses, thin-walled except for apex ..... *Sphaeriales*
               10. Asci within a wall layer of *vertical hyphae* (pseudoparaphyses) connected in roof of perithecium, with wall uniformly thickened ..... *Hypocreales*
            9. Asci in a *uniloculate* or *multiloculate stroma*, opening by an apical lysigenously formed pore; with no periphyses and no differentiated wall to the ascal centrum. Ascal wall uniformly thickened, with *no pore, opening by the outer wall splitting and the inner protruding.*

11. Ascocarp opening by a circular pore.
12. Asci in basal fascicle in the stroma with no interthecial threads ..... *Dothideales*
12. Asci with pseudoparaphyses connected at top and bottom of locule.
13. Hymenium in wall-layer, ascocarp variously shaped, not dimitate *Pseudosphaeriales*
13. Hymenium in flat, basal layer, ascocarp dimitate ..... *Microthyriales*
11. Ascocarp opening by an elongate slit, pseudoparaphyses in locule.
14. Ascocarp perithecial-like ..... *Lophiostomatales*
14. Ascocarp boat-shaped ..... *Hysteriales*

4. Ascocarp open. Hymenium either exposed from the first, or inclosed and later becoming more or less widely exposed, or if opening only by weathering then hypogeic; paraphyses apically free; ascocarps variously shaped apothecia ..... DISCOMYCETES
15. Asci inoperculate, without a definite method of opening or opening by a pore.
16. Asci cylindrical, apically obtuse with thickened membrane penetrated by a canal, with filiform spores; spores septate, breaking into cylindrical parts ..... *Ostropales*
16. Asci and ascospores not as above ..... *Helotiales*
15. Asci operculate, opening by a lid.
17. Ascocarp epigeic at least at maturity, hymenium exposed from the first or inclosed and later opening ..... *Pezizales*
17. Ascocarp hypogeic, hymenial layer exposed by weathering *Tuberales*

A KEY TO THE SPHAERIALES

1. Ascus with thickened apical cap, penetrated by a narrow, threadlike pore; spores filiform and breaking into segments ..... *Clavicipitaceae*
1. Ascus without cap, pore not as above, spores of various shapes, if filiform, not breaking into segments.
2. Asci very thick at apex, pore well defined.
3. Inner wall of ascus producing a crown at base of pore, with latter ending in a wide plug. Asci long stalked, persistent; paraphyses persistent, filiform and branched; ascospores 1-celled, dark, germinating by a longitudinal germ pore, uniseriate *Xylariaceae*
3. Crown not present, a light-refractive ring in pore.
4. Asci long stalked, persistent, paraphyses persistent, filiform and branched; ascospores chiefly 1-celled, allantoid; ostiola sulcate ..... *Allantosphaeriaceae*
4. Asci short-stalked, not persistent, soon floating loose in cavity of perithecium, spores of various shapes, paraphyses as broad bands, gelatinizing at maturity; ostiola not sulcate *Diaporthaceae*

2. Asci not especially thickened at apex, wall of uniform diameter, with pore not very evident.
   5. Fungi in living leaves, with a stroma, or only forming a darkened clypeus by proliferation of ostiolar tissue; perithecia within leaf, with pseudoprosenchymatous walls; ascospores uniseriate; paraphyses copious and persistent..... *Phyllachoraceae*

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EXPLANATION OF FIGURES

FIG. 1. Asci of *Taphrina caerulescens*. FIG. 2. *Myriangium Duriaei*, single globose ascus. FIG. 3. *Dothiora subtropica*, asci in stroma, representing high point in Myriangiales with asci in single layer and tending toward the cylindric. FIG. 4. *Microsphaera Alni*, semi-globose ascus. FIG. 5. Longitudinal section of perithecia of *Cordyceps militaris*, showing definite wall. FIG. 6. Cross section of fertile stroma of *Claviceps purpurea*, also showing walls to perithecia. FIG. 7. Longitudinal section of *Claviceps purpurea*. FIG. 8. Ascus of *Cordyceps capitata*, showing cap and narrow pore. FIGS. 9, 10. Asci of *Hypoxylon tinctor* and *Xylaria Hypoxylon*, showing crown and wide pore in apices. FIG. 11. Asci of *Eutypella fraxinicola*, showing stalks and refractive ring in pores. FIGS. 12, 13, 14 and 15. Asci of *Valsa ambiens*, *Diaporthe oncostoma* (13, 14) and *Cryptospora cintula*, all showing the lack of stalk, and the refractive ring in the pore. FIG. 16. Young perithecium of *Phyllachora Lespedezae*, showing definite wall,

free paraphyses and asci growing into a cavity. FIG. 17. Perithecium of *Phyllachora graminis*, showing the wall with no stroma, and asci and free paraphyses. FIG. 18. Young perithecium of *Nectria coccinea*, showing vertical threads attached at top and bottom and a wall layer of very young asci. FIG. 19. Mature perithecium of *Hypocrea lenta*, showing the remnants of the vertical threads just before gelatinization. FIG. 20. A medium aged perithecium of *Nectria coccinea*, showing attached vertical threads and young asci. FIG. 21. *Nectria galligena*, showing asci with pores and wide plugs. FIGS. 22–23. *Auerswaldiella puccinioides*. Immature locule of figure 22 shows vertical threads and asci in or at their bases, while figure 23 is a mature locule with the attached threads still showing. FIG. 24. Single ascus locule of *Dibotryon morbosum*, showing attached threads between the asci. FIG. 25. *Lasiosphaeria ovina*, also showing attached threads. FIG. 26. An ascus of *Botryosphaeria Ribis*, with thick wall and no apical pore. FIG. 27. An ascus centrum of *Pleospora aurea*, showing asci with thick walls and no pores and the vertical threads. FIG. 28. Asci of *Massaria Argus*, also showing thick walls and no pores. FIG. 29. *Fracchiaea heterogena*. FIG. 30. *Coronophora ootheca*. FIG. 31. *Cryptosphaerella annexa*. All three asci have allantoid spores, but the walls are not thickened at the apices and there is no pore. FIG. 32. A multiloculate stroma of *Auerswaldia examinans* with asci growing up in the stroma with no paraphyses nor vertical threads. FIG. 33. *Dothidea collecta*, showing the same locule centrum characters as figure 32. FIG. 34. Asci of *Dothidea collecta*, showing uniformly thickened walls with no pores, and the basal attachment producing the fascicle characteristic of the order. FIG. 35. *Mycosphaerella effigurata*, showing the same type of locule centrum as in the *Dothidea* species. FIG. 36. Ascus of this *Mycosphaerella*, also showing characters typical of those of the *Dothidea*. FIG. 37. Ascus of *Hysterographium flexuosum*, showing the uniformly thickened wall and no pore.

THE "DUAL PHENOMENON" AND TRICHOPHYTON MENTAGROPHYTES [1]

WILLIAM J. ROBBINS AND ILDA MCVEIGH

(WITH 1 FIGURE)

Wilhelm (3) has applied the concept of "dual phenomenon" developed by Snyder and Hansen to the dermatophytes. He assumes that a species of a dermatophyte is composed of races and each race is dual, that is, composed of two distinct constituents associated together in culture. One constituent produces conidia in abundance but relatively scanty mycelium; this he designates as the C or conidial constituent. The other produces fewer conidia but more abundant mycelium; this he designates as the M or mycelial constituent. The M type, he says, arises as a mutation in old cultures of the C type even though the culture is started from a single conidium.

In addition to the C and M constituents, a "race" of a dermatophyte may give rise to forms culturally intermediate between C and M. Wilhelm suggests that in old cultures the mycelium and spores become heterocaryotic with respect to C and M nuclei. The variation exhibited upon single spore analysis of these old cultures results from chance separation of nuclear types in the spores at the time they are formed. He concludes that the normal and pleomorphic forms of *Trichophyton mentagrophytes* pictured by Robbins and McVeigh (2) may be considered respectively as C and M types.

We interpret Wilhelm's suggestion to mean that given a race of a dermatophyte in the conidial form, the following occurs: The conidial type, C, mutates and forms the mycelial type, M. Heterocaryosis occurs between the C and M, that is, mycelium develops

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containing both C and M nuclei; this form is intermediate in character between the C type and the M type. If single spore isolations are made from the C type, only C is obtained because only C nuclei were present. Similarly, only M is obtained from M. However, single spore isolations from intermediate forms, CM, yield the conidial type, the mycelial type, and, in addition, intermediates if the spores isolated are multinucleate.

Our experience with the variants which occur in older cultures of *T. mentagrophytes* has led us to doubt that the concept of a "dual phenomenon" supplemented with heterocaryosis and the separation of nuclei is an adequate explanation for the occurrence and characteristics of the variants which can be isolated from old cultures of this fungus. In the first place, the number of variants which can be isolated from a single "race" appears to us to be greater than can be accounted for by heterocaryosis. In the second place, we have found that the variants can be maintained indefinitely in culture provided transfers are made to fresh media at relatively short intervals—a week or thereabouts. If any of the variants isolated from old cultures of *T. mentagrophytes* were heterocaryotic, we would scarcely anticipate that they could be maintained in culture without evidencing nuclear separation since many of them form spores freely before subcultures are made.

The application of the concept of "dual phenomenon" to *T. mentagrophytes* was tested experimentally by isolating nine variants from an original conidial culture which had been obtained from a human subject. Colonies of the nine variants and the original form grown on peptone agar and asparagine agar are shown in figure 1. They ranged from a highly mycelial rapidly-growing form which produced conidia slowly to highly conidial slow-growing forms. Since these variants were obtained from old cultures of the conidial form on which pleomorphic colonies had developed, it would seem justifiable to assume that some of the isolations were intermediates, as defined by Wilhelm.

Each of the ten isolations was maintained at approximately 35° C. on peptone agar by making transfers to fresh media at short intervals. Single spore isolations from each were made by spreading spores thinly on the surface of an agar plate. After one or

two days incubation, individual microconidia which had begun to germinate were selected (using a binocular or the lower power of a compound microscope), and transferred to tubes. Comparisons were made of the resulting colonies.

A description of the origin of each of the isolations and their general characteristics, together with the results obtained on isolations of single microconidia, is given below.

Several media were used as follows. Asparagine-peptone agar contained per liter 50.0 g. dextrose, 1.5 g. KH_2PO_4, 0.5 g. $MgSO_4 \cdot 7H_2O$, 1500 mμ moles thiamine, 2.0 g. asparagine, 1.0 g. neopeptone, 0.5 ml. mixture of mineral supplements (1), and 15.0 g. agar. Peptone agar was prepared as above except that it contained 2.0 g. per liter of neopeptone and no asparagine. Asparagine agar was the same as the asparagine-peptone agar with peptone omitted. Ammonium nitrate agar was identical with the asparagine-peptone agar except 2.0 g. of NH_4NO_3 was substituted for the asparagine and peptone. The pH of these media ranged between 5.0 and 5.6.

The morphology of the colony, pigment production, conidial formation and microscopic character of a particular isolate are affected by the composition of the medium and by the temperature and time of incubation. In our experience, the distinctions between isolates, at least so far as colony appearance is concerned, are sharper if the medium is not too rich. We have, therefore, made our comparisons on media containing relatively small amounts of nitrogen.

The (N) form

T. mentagrophytes was originally isolated from a human subject in August, 1942. It was a freely sporulating form with sparse mycelial growth corresponding to the condial type described by Wilhelm. We have referred to this as the normal (N) strain. A single spore culture was made and maintained in a freely sporulating condition up to April 24, 1947, on asparagine-peptone agar by subculturing to fresh media at intervals which did not exceed two weeks. Since the date given, it has been maintained on peptone agar by transferring at weekly intervals to fresh media.

Microscopic examinations of cultures of (N) grown on peptone agar at 35° C. made at the end of one and two weeks, showed the mycelium to be variable; both slender and wide hyphae occurred. Microconidia were numerous in one week. Chains of chlamydospores were common. No macroconidia were observed in two weeks on this medium.

The (N) form grows slowly on an asparagine medium, more rapidly on peptone. Little or no growth occurs with NH_4NO_3 as source of nitrogen.

When single spore isolations were made from the (N) form, they developed into (N) cultures.

In February, 1946, sixty single spore isolations from a young culture of (N) were made to asparagine-peptone medium. Subcultures of each isolation were made to NH_4NO_3 agar. The isolations on asparagine-peptone agar were indistinguishable when examined after seven and nine days, and those on NH_4NO_3 gave characteristic response for (N) on that medium.

On May 3, 1948, sixty-nine single spore isolations were made to peptone agar from a three-day old (N) culture which had grown on peptone agar at 35° C. All proved to be identical in appearance when examined after ten days. Subcultures of each isolation were made after four days to an asparagine medium. All appeared to be alike after six days growth.

If cultures of the (N) strain are allowed to age, variants develop which can be distinguished by rate of growth, morphology of colony, pigment production, microscopic appearance and physiological characteristics. Some of these have been described earlier (**1**, **2**). Although no particular effort has been made to discover how many variants could be isolated, it is our impression that they would be considerable in number—fifty or more. In any event, those which we have isolated have been maintained in culture apparently unchanged over considerable periods by transferring to fresh media at intervals of a week or thereabouts.

The (B) variant

A strongly mycelial type was isolated by mass transfer from a pleomorphic colony which had developed on an (N) culture. A

single spore isolation was made and labeled (B). It has been maintained in culture for over three years.

In February, 1946, forty-six single spore isolations of (B) were made to asparagine-peptone agar. Subcultures of each of the isolations were made to NH_4NO_3 agar. All isolations on peptone agar appeared to be alike, and the subcultures to NH_4NO_3 agar were indistinguishable one from another.

On May 5, 1948, sixty-five single spore isolations were made from a thirteen-day old culture of (B) to a peptone medium, and subcultures were made from each isolation after six days to an asparagine medium. Examinations made up to twelve days on the peptone and six days on the asparagine medium showed all isolations to be indistinguishable.

The (B) form is the most rapidly growing form we have isolated. On peptone and asparagine agar it produces a thick, white, fluffy colony. It is able to use NH_4NO_3 though growth is less rapid than with asparagine or peptone.

Microscopic examination of cultures grown at 35° C. on the peptone medium were made at the end of one, two, three and four weeks. At the end of one week, the hyphae were slender and uniform in width. No micro- or macroconidia and no chlamydospores were observed. A few hyphae had swollen tips. On the older cultures, some thicker hyphae were noted and some of the slender hyphae developed thickened portions, but no chains of chlamydospores were found. A few microconidia had developed by the end of two weeks and the number increased until they were numerous after four weeks. A few macroconidia were observed in cultures four weeks old.

The (A) variant

A mycelial type was isolated from a pleomorphic colony on a plate inoculated with (N). A single spore isolation was made and the resulting culture labeled (A). It was less vigorous than (B). It produced conidia more freely than (B) but much less so than (N). The hyphae were variable in diameter but the proportion of wide hyphae was less than in cultures of (N). Macroconidia were uncommon up to four weeks and no chains of chlamy-

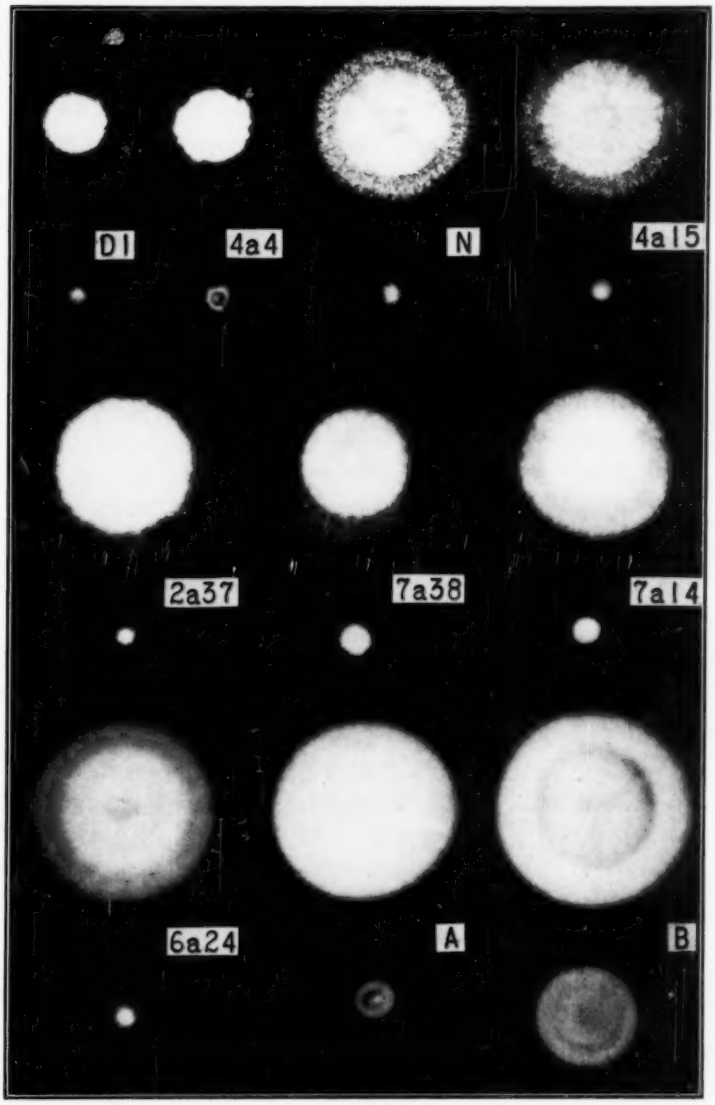

FIG. 1. A conidial race (N) of *Trichophyton mentagrophytes* and nine variants derived from it grown at 35° C. for twelve days on peptone agar and eleven days on asparagine agar. For each strain the growth on asparagine agar is below; on peptone agar, above.

dospores were observed though occasional swellings in the hyphae occurred.

In January, 1948, 218 single spore isolations of (A) were made to asparagine agar from seven-, eight- or nine-day-old cultures on peptone agar. After seven or eight days, transfers were made to peptone agar. All cultures appeared to be alike. Transfers were made from the peptone cultures to an asparagine medium. One week later all cultures looked alike. None resembled (N) or (B).

On April 29, 1948, fifty-six single spore isolations were made to peptone agar from an (A) culture which had grown seven days at 35° C. on peptone agar. All these isolations appeared alike up to fourteen days. Transfers were made to asparagine agar from each isolation after six days on peptone agar. When examined six days later, all cultures looked alike.

Variant (6a24)

A mass isolation was made from a pleomorphic area appearing on an agar plate nine days after inoculation with a spore suspension from a seven-day-old culture of (N). This was labeled (6a) and thirty-six single spore isolations were made on peptone agar. All of these appeared alike after eleven days. One of these isolations, (6a24), was selected for further study. On May 10, 1948, sixty-five single spore isolations of (6a24) were made from a four-day-old culture to peptone agar and after four days, subcultures of each were made to an asparagine agar. Observations made after eleven days growth on the peptone agar indicated that all of these cultures were alike in gross morphology. The subcultures on the asparagine medium appeared alike after one week.

Microscopic examination showed that this isolation resembled form (B) in being composed of slender hyphae. However, in the production of conidia (6a24) resembled the (N) form. Microconidia were very numerous in a culture grown on peptone agar for one week. Chains of chlamydospores were uncommon.

Macroscopically, (6a24) on peptone agar was much like the (A) and the (B) form. It consisted of a rapidly spreading, thick, aerial mycelium. Its growth characteristics on an asparagine medium, however, were different from those of any of the forms studied.

On this medium, it formed a small central area of heavy mycelium with a wide area of sparse subsurface mycelium.

Variant (7a38)

A mass isolation from a pleomorphic colony appearing on a plate originally inoculated with a seven-day-old culture of (N) was labeled (7a). The cultures which developed from thirty-two single spore isolations made on peptone agar were of two types. Both types were faster growing and less powdery than the (N) form. They resembled the (A) and (B) forms in being fluffy. One type produced considerable yellow pigment in the peptone agar and formed a small powdery area at the upper area of the slant. Twenty-one of the isolations were of this type, and a single one, (7a38), was selected for further study. The remaining eleven isolations were fluffy over the entire surface and developed little or no pigment. This type spread less rapidly than the first described. One of this form, (7a14), was reserved for further study.

On May 6, 1948, seventy single spore isolations were made from a six-day-old culture of (7a38) to a peptone medium. Minor differences in pigmentation and colony size were noted when the cultures were first observed. Observations after eleven days showed all to be of one type. Subcultures of each of the isolations were made to asparagine agar five days after isolation. On the asparagine medium, all were alike after six days.

Microscopically, (7a38) resembled the (N) form. The mycelium was composed of both slender and wide hyphae. Both microconidia and chains of chlamydospores were present in a week-old culture. The conidia were numerous.

(7a38) on plates of peptone agar was intermediate between the (N) and (B) forms in appearance. It formed a heavier growth than (N) but less than (7a14). On the peptone agar, the most distinguishing characteristic was the production of yellow pigment. It grew slightly more rapidly on the asparagine agar than (7a14).

Variant (7a14)

On May 10, 1948, seventy single spore isolations were made from a five-day-old culture of (7a14) to peptone agar. After ten

days, all seventy of the isolations appeared alike. Six days after isolation, subcultures of each were made on asparagine agar. Observations made six days later indicated that all were alike in gross morphology.

The mycelium of (7a14) was composed of very slender hyphae. Microconidia in a week-old culture were more numerous than in the (A) form. There were occasional swollen hyphae similar to those observed in the (A) form. No chains of chlamydospores were observed.

On peptone agar plate cultures, (7a14) in general appearance was much like the (B) form except that it did not spread so rapidly. On asparagine plates, (7a14) grew less rapidly than (B). It formed a small area of dense growth with thin subsurface radiations.

Variant (2a37)

A mass isolation from a pleomorphic colony on a plate originally seeded with (N) was labeled (2a). Fifty-five single spore isolations from this culture made on peptone agar appeared to be alike after nine days growth. One of these, (2a37), was selected for further study. Sixty-eight single spore isolations were made to peptone agar from a five-day-old culture of (2a37) on peptone agar. All appeared to be alike after thirteen days growth. Transfers of each isolation were made to asparagine agar after six days on the peptone agar. All appeared to be alike after six days growth on the asparagine agar. (2a37) was less vigorous on peptone agar than (B), (A), (6a24), (7a14) or (7a38). Growth was heavier and less powdery than (N).

Microscopic examinations of (2a37) were made after one and two weeks growth at 35° C. on peptone agar. The hyphae were variable in width; the thicker hyphae predominated at the end of two weeks. Microconidia were numerous at the end of one week. Chains of chlamydospores were rare at the end of one week and more numerous but not common after two weeks. Macroconidia were infrequent.

Variant (4a15)

A mass isolation made from a pleomorphic colony which had developed on a plate originally seeded with (N) was labeled (4a). Thirty-two single spore isolations were made from this culture to peptone agar. Two types of colonies were distinguishable, both powdery and in general resembling (N). One type grew somewhat more rapidly on peptone agar than (N); the other less rapidly. A single isolation, (4a15), of the first group, and one, (4a4), of the second group, were selected for further study.

On May 10, 1948, sixty-one single spore isolations were made to peptone agar from a culture of (4a15) which had grown for five days at 35° C. on peptone agar. After fourteen days, all isolations appeared to be alike. Transfers were made to asparagine agar from each isolation at the end of seven days. After seven days, all cultures on asparagine agar appeared alike.

Macroscopically and microscopically, (4a15) resembled (N) closely. It is distinguishable on asparagine agar where the growth is less powdery and heavier than (N).

Variant (4a4)

On May 7, 1948, sixty-eight single spore isolations were made to peptone agar from a culture of (4a4) which had grown for six days at 35° C. on peptone agar. On examination twelve days later, sixty-five isolations appeared to be alike and three seemed somewhat more fluffy. Subcultures of these three and three of the others were made to peptone agar and proved to be indistinguishable. After six days, transfers of each isolation were made to asparagine agar. Six days later, all cultures on asparagine agar appeared to be alike.

Form (4a4) was less vigorous on peptone agar than (N) but somewhat more so than (D1). Microscopic examinations were made of cultures grown on peptone agar at 35° C. after one and two weeks. The hyphae were variable but wide ones predominated. Microconidia and chains of chlamydospores were numerous. Macroconidia were not observed.

Variant (D1)

A strongly conidial type, slower growing than (N), was isolated from plates of asparagine-peptone agar which had been inoculated from a seven-day culture of (N) and incubated for fifty-four days at 35° C. Evident pleomorphisms had developed on this plate. A spore suspension was made and plated on asparagine-peptone agar. Three types of colonies developed—namely, pleomorphic forms, (N) forms, and some slower growing than (N). An isolation was made May 1, 1946, from one of the slow-growing colonies and labeled (D). A single spore isolation was made from (D) and labeled (D1). This variant has been maintained in culture to date by making transfers to fresh media at seven-day intervals. On May 5, 1948, seventy-eight single spore isolations were made to peptone from a five-day-old (D1) culture growing on peptone agar. All seventy-eight isolations proved to be identical in gross morphological appearance when examined after twelve days. After six days on peptone, transfers from each of the seventy-eight isolations were made to asparagine agar. All were identical in appearance when examined after six days incubation. When first examined, six of the subcultures on asparagine agar appeared slightly more fluffy than the balance. Transfers of these six and some of the other seventy-two were made to peptone agar and were found to be indistinguishable. We concluded that all the single spore isolations of (D1) were alike.

DISCUSSION

It seemed to us that the concept of the "dual phenomenon," as we interpret its application by Wilhelm to dermatophytes, could be tested by selecting a number of variants which had developed in old cultures of the condial form of a "race" of *T. mentagrophytes*. Evidence on the existence of heterocaryosis and nuclear separation could be obtained by comparing cultures derived by single spore isolations from young cultures of these variants.

Since our strain of *T. mentagrophytes* was isolated from a human subject and developed from a single microconidium, we considered it to be a single "race." The variants were all obtained from this "race."

Some of the variants selected were intermediate between the ex-

treme mycelial type and the pronounced conidial form in growth habit, conidial production and microscopic appearance. We considered that at least some, if not all, of these would be intermediates in Wilhelm's meaning and therefore heterocaryotic. If this assumption were correct, then both the mycelial and conidial types would be obtained by single spore isolations from these intermediates. If the spores isolated were uninucleate, these two types only would be observed. If the spores or other reproductive bodies were multinucleate, a few of the conidial and a few of the mycelial types might be expected, but various kinds of intermediates, depending upon the relative proportions of C and M nuclei in the spores isolated, would predominate. Young cultures only could be used for single spore isolations because of the possibility that mutants had developed in the older cultures.

Our results were entirely negative. In no instance did we obtain a conidial or a mycelial type from the microconidia isolated from young cultures of forms which appeared to be intermediate between the two types. In every instance the cultures which grew from single microconidia were indistinguishable from the parent.

The number of conidia from each isolation with which we dealt was not large. We believe, however, that it was sufficiently large to detect nuclear separation, though too small to discover spontaneous mutants which might be assumed to occur at the rate of 1 to 10,000 or of that order.

It seems unlikely that Wilhelm's suggestion is adequate to explain the variants which develop from a single strain of *T. mentagrophytes*. If the variants with which we worked were derived from multinucleate spores, we should have observed nuclear separation. If they originated from spores which were uninucleate, they should have been either conidial or mycelial but not intermediate.

We are of the opinion that the variants which develop, as cultures of *T. mentagrophytes* age, are mutations, though whether the change is cytoplasmic or nuclear cannot be determined because of the absence of a sexual stage.

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THE SWARM-CELLS OF MYXOMYCETES

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(WITH 25 FIGURES)

Germination of spores of a species of myxomycete was first reported by deBary (**1**) in 1854. He observed that the spores of *Hemitrichia Vesparium*[1] produced a flagellated swarm-cell rather than the hyphal tube characteristic of the higher fungi, with which the Myxomycetes were then classified. Subsequently deBary (**3**, **4**) attempted to germinate spores of a number of species, meeting with varying success. He obtained no germination of spores of the *Cribrarias* and the *Tubiferas*.

Since deBary's first report, many students of the Myxomycetes have attempted to germinate a large number of forms and have invariably met with the same inconsistencies which deBary found. Durand (**13**) attempted to germinate several species, succeeding only with *Enteridium Rozeanum*. McClatchie (**43**), reading of Durand's low results, replied with a statement that he and his students never failed to obtain good germination with spores of *Reticularia Lycoperdon* whether the collections were fresh or a year old. He germinated five other species with varying results, but always obtained swarm-cells within three hours except in *Hemitrichia Vesparium*, which produced only myxamoebae. Lister (**40**) found that repeated drying and rewetting of cultures facilitated the germination of a species of *Badhamia*. Jahn (**29**) used this method to obtain germination of *Stemonitis axifera* after previous culturing had failed, and later (**30**) used it to obtain germination—in very low numbers, to be sure—of *Tubifera ferruginosa* and *Lindbladia effusa*. Both of these belong to families with which deBary had had no success. All reports since Jahn's confirm deBary's experience with these two species. F. A. Gilbert (**21**) made a comparative study of spore germination by families.

[1] Nomenclature throughout this paper follows the classification according to Martin (**42**). Where current names differ from those used by the authors of the papers cited, the synonomy is given in the appendix.

He included reports in the literature as well as results obtained with his own collections of fifty-six species. He reports that all of the Reticulariaceae germinated very well, and that the *Tubiferas* had not ever been germinated, apparently overlooking Jahn's report. He found that in the other families tested "germinative ability appears to differ with the genera and species."

Studies of the factors influencing spore germination have brought out many points but have left many others to be clarified. McClatchie reported a higher percentage of germination in *Reticularia Lycoperdon* one year old than in fresh collections. Gilbert reported no germination of *Lycogala* less than one month after fruiting, but good germination of older material. He found that fresh collections of *Leocarpus fragilis* germinated only after two and a half days in culture while older spores germinated in twelve hours. E. C. Smith (**55**) germinated twenty-one collections ranging from five to thirty-two years old and found no correlation between the ages of his collections and their percentages of germination. Other workers have observed improvement of germination with aging within relatively short limits of time. Gilbert regarded the aging of the spores as important in their maturation and listed this as one of the most important factors governing germination. He also referred to "internal factors" varying from species to species.

Smart (**52**) studied the influence of some external factors on the germination of about seventy species. He found that most forms germinate better, though usually little better, in decoctions of their natural substrata than in distilled or tap water. The optimum temperature for all forms studied was between 22° and 30° C., most forms doing better near 30° C. than at lower temperatures. The optimum pH ranged from 4.5 to 7.0, most forms germinating most readily near neutrality. It is important to note that the variation in percentage germination of any given species resulting from variation of any one factor is less than the differences in percentage germination among the various species even though all factors be optimum for each species. In other words, Smart's intensive study of external factors serves to bring out the point that the factors he studied are of less importance in spore germination than some other factor or factors not yet discovered.

Several workers have stressed the necessity of stirring the spores

into the culture solution to assist in wetting. Cayley (**9**) used alcohol as a wetting agent to increase germination of spores of *Didymium nigripes*. Smart (**52**) disinfected spores with mercuric chloride or hexylresorcinol, followed by washing with centrifuging and final culturing in distilled water. Though his objective was reduction of bacteria, probably the manipulation had considerable wetting action, and many of the side effects which he observed might be attributable to increased numbers of spores thoroughly wetted.

Gilbert (**18**) and Smith (**54**) both reported that approximately half of the species which they observed produced more than one swarm-cell per spore. Gilbert observed the production of one, two, or four protoplasts from spores of *Leocarpus fragilis*, and Smith states that he observed four protoplasts escape from each of fifty spores of *Badhamia affinis* before seeing a single protoplast emerge from one spore. Howard (**26**) says spores of *Physarum polycephalum* may produce one or two swarm-cells, and Heitzmanowna (**24**) observed "one to several" swarm-cells produced by spores of *Didymium nigripes*.

Cultures of swarm-cells of Myxomycetes are ordinarily contaminated by bacteria, and usually by any or all of several species of protozoa. DeBary pictures a protozoan with a flagellum at each end among his swarm-cells. Durand observed this same form and considered it an abnormal form of swarm-cell characteristic of *Enteridium Rozeanum*. Pinoy (**45**, **46**) considered certain bacteria and protozoa necessary for germination and development of Myxomycetes and even postulated that the protozoan cysts are incorporated into myxomycete fructifications. Gilbert studied the bipolar flagellate and reported it as *Cercomonas longicauda*.

Anteriorly biflagellate swarm-cells have been observed as occurring occasionally in Myxomycetes by many observers, but it has been generally held that the swarm-cells in this group are normally uniflagellate. DeBary (**4**) in 1884 and Vouk (**58**) in 1911 reported biflagellate forms in exceptional cases only. Gilbert (**16**) found one-fourth of the swarm-cells of *Stemonitis fusca* biflagellate. Von Stosch (**56**) saw biflagellate cells in many other species, but none in the single species of *Stemonitis* which he studied. Gilbert (**17**) reported *Dictydiaethalium plumbeum* to be uniflagel-

late, but E. C. Smith (**54**, **55**) twice within the following year published photomicrographs showing biflagellate swarm-cells in the same species. Howard (**26**) termed biflagellate swarm-cells in *Physarum polycephalum* "common." Sinoto and Yuasa (**49**) studied *Ceratiomyxa* and four species of Myxogastres, finding only one flagellum in *Ceratiomyxa*, but occasional bi- and even tri-flagellate forms in all of the others. Yuasa (**60**) likewise found bi- and tri-flagellate cells in *Fuligo septica*. Jahn (**31**) in 1928 ignored the previous reports of biflagellate swarm-cells, and in 1936 (**32**) he criticized the work of Von Stosch, insisting that swarm-cells are normally uniflagellate and that all biflagellate swarm-cells are anomalies. Karling (**35**), in his general summary of the literature pertinent to the relationships between the Plasmodiophorales and the Myxomycetes, says, ". . . although the majority are uniflagellate, zoospores with two flagella are not uncommon. . . ." In 1945 Ellison (**15**) reported biflagellate swarm-cells in proportions varying from two per cent to twenty-six per cent for a number of species of Myxomycetes but retained the assumption that the majority are uniflagellate.

It is not inconceivable that an occasional biflagellate swarm-cell could occur as an abnormality in an otherwise uniflagellate group. But biflagellation has already been reported too frequently in the Myxomycetes to represent mere abnormality. And on the basis of flagellation as reported in other groups, it is very unlikely that both uniflagellate and biflagellate forms would normally exist in the same life stage of the Myxomycetes. It seems more probable that the second, shorter, flagellum is difficult to see and is frequently hidden.

The purposes of this study were to find a means of improving germination *in vitro* of myxomycete spores, and to study the swarm-cells thus produced in an effort to determine whether anterior biflagellation is not the rule rather than the exception.

METHODS AND MATERIALS

Ninety collections representing fifty-nine species in thirty genera were used for these tests. Following is a list of the collections used, with the place and year of collection.

Ceratiomyxales		
Ceratiomyxaceae		
Ceratiomyxa fruticulosa (Muell.) Macbr.	1. Canal Zone	1945
	2. Minnesota	1946
	3. Iowa	1948
Liceales		
Liceaceae		
Tubifera ferruginosa (Batsch) J. F. Gmel.	4. Iowa	1934
	5. Montana	1935
	6. Illinois	1947
Reticulariaceae		
Dictydiaethalium plumbeum (Schum.) Rost.	7. Iowa	1947
	8. Iowa	1947
Enteridium Rozeanum (Rost.) Wing.	9. Ohio	1893
	10. Iowa	1946
	11. Iowa	1946
	12. Iowa	1947
Lycogala epidendrum (L.) Fries	13. Indiana	1944
	14. Iowa	1946
	15. Iowa	1947
Reticularia Lycoperdon Bull.	16. Iowa	1929
	17. Iowa	1942
	18. New York	1947
	19. Iowa	1947
Cribrariaceae		
Cribraria intricata Schrad.	20. Nebraska	1894
	21. Illinois	1940
Cribraria purpurea Schrad.	22. West Virginia	1947
Dictydium cancellatum (Batsch) Macbr.	23. Iowa	1942
Lindbladia effusa (Ehrenb.) Rost.	24. South Dakota	1910
Trichiales		
Trichiaceae		
Arcyria cinerea (Bull.) Pers.	25. Canal Zone	1945
Arcyria denudata (L.) Wettst.	26. Iowa	1947
Arcyria incarnata Pers.	27. Iowa	1946
Hemitrichia clavata (Pers.) Rost.	28. Iowa	1946
Hemitrichia Serpula (Scop.) Rost.	29. Kentucky	1945
Hemitrichia stipitata (Massee) Macbr.	30. Kentucky	1946
Hemitrichia Vesparium (Batsch) Macbr.	31. West Virginia	1947
Oligonema Schwenitzii (Berk.) Martin	32. Iowa	1947
	33. Iowa	1947
	34. Iowa	1947
Perichaena chrysosperma (Currey) Lister	35. Kentucky	1947
Perichaena depressa Libert	36. Georgia about	1940
Trichia affinis deBary	37. Iowa	1946
Trichia favoginea (Batsch) Pers.	38. Iowa	1947
Trichia varia (Pers.) Pers.	39. California	1947

Stemonitales			
Echinosteliaceae			
Echinostelium minutum deBary	40.	Louisiana	1936
	41.	Iowa	1937
	42.	Iowa	1943
	43.	Indiana	1945
	44.	Minnesota	1946
	45.	Rhode Island	1947
Stemonitaceae			
Clastoderma Debaryanum Blytt	46.	Rhode Island	1947
Comatricha laxa Rost.	47.	Iowa	1945
Comatricha Suksdorfii Ellis & Ev.	48.	California	1947
Comatricha typhoides (Bull.) Rost.	49.	Iowa	1937
	50.	Minnesota	1947
Diachaea bulbillosa (Berk. & Br.) A. Lister	51.	Canal Zone	1945
Enerthenema melanospermum Macbride & Martin	52.	California	1946
Enerthenema papillatum (Pers.) Rost.	53.	Indiana	1945
	54.	Illinois	1947
Lamproderma arcyrionema Rost.	55.	Illinois	1947
Lamproderma Sauteri Rost.	56.	California	1946
Lamproderma scintillans (Berk. & Br.) Morgan	57.	Iowa	1942
Stemonitis axifera (Bull.) Macbr.	58.	Maryland	1894
	59.	Michigan	1935
	60.	Indiana	1944
	61.	Kentucky	1946
	62.	California	1947
Stemonitis flavogenita Jahn	63.	West Virginia	1947
Stemonitis fusca Roth.	64.	Iowa	1927
Stemonitis splendens Rost.	65.	Iowa	1947
Physarales			
Physaraceae			
Badhamia gracilis Macbr.	66.	Panama	1935
Badhamia panicea (Fries) Rost.	67.	Iowa	1938
Cienkowskia reticulata (Alb. & Schw.) Rost.	68.	Louisiana	1948
Craterium leucocephalum (Pers.) Ditm.	69.	Iowa	1941
	70.	Illinois	1947
Fuligo septica (L.) Wiggers	71.	Indiana	1944
	72.	Michigan	1947
Leocarpus fragilis (Dicks.) Rost.	73.	Iowa	1946
Physarella oblonga (Berk. & Curt.) Morgan	74.	Kentucky	1947
Physarum cinereum (Batsch) Pers.	75.	Iowa	1939
Physarum contextum (Pers.) Pers.	76.	Illinois	1947
Physarum didermoides (Pers.) Rost.	77.	Illinois	1947
Physarum flavicomum Berk.	78.	Pennsylvania	1886
Physarum globuliferum (Bull.) Pers.	79.	Iowa	1946
Physarum melleum (Berk. & Br.) Massee	80.	Hawaii	1946
Physarum nutans Pers.	81.	Iowa	1942
Physarum polycephalum Schw.	82.	Iowa	1947
Physarum viride (Bull.) Pers.	83.	Rhode Island	1945

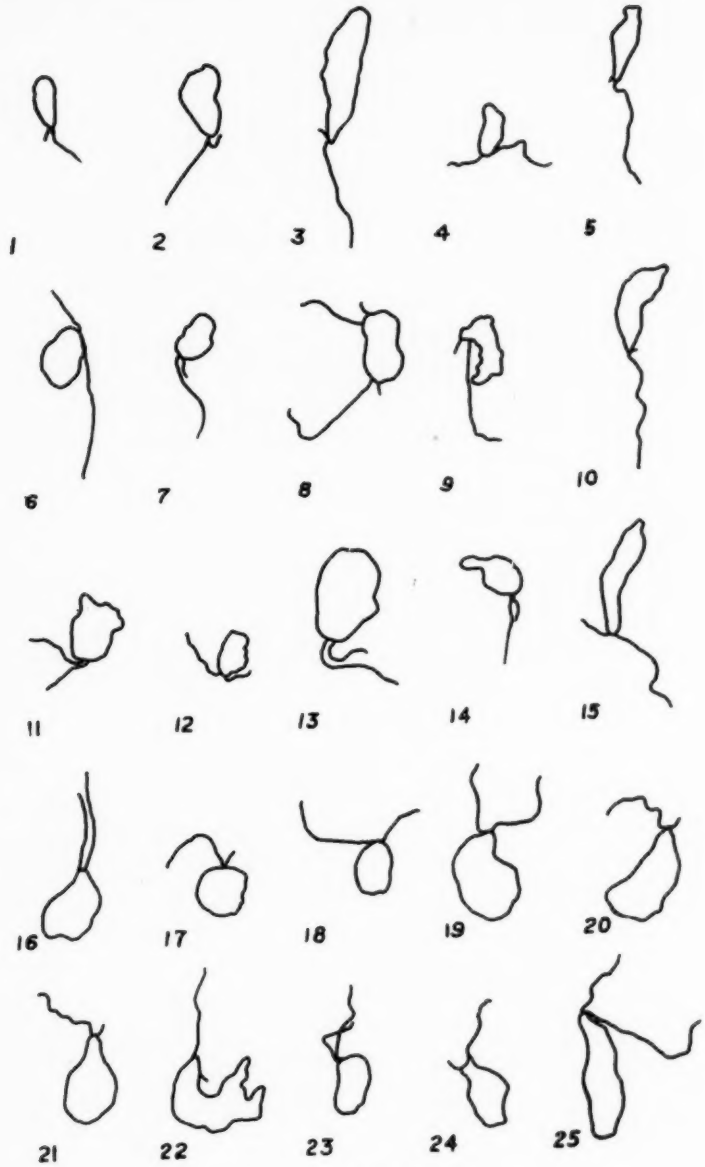
Didymiaceae		
Diderma floriforme (Bull.) Pers.	84. Iowa	1947
Diderma globosum Pers.	85. Iowa	1947
Didymium squamulosum (Alb. & Schw.) Fries	86. Minnesota	1946
	87. Iowa	1947
	88. Iowa	1948
Didymium xanthopus (Ditm.) Fries	89. Iowa	1948
Mucilago spongiosa (Leysser) Morgan	90. Indiana	1944

The first cultures were prepared in September, 1946, using *Reticularia Lycoperdon* (Coll. no. 16, above), *Lycogala epidendrum* (no. 14), and *Trichia affinis* (no. 37). Abundant germination was obtained in the first attempts with *R. Lycoperdon* and *T. affinis*, but only one of several cultures of *L. epidendrum* was observed to germinate. Repeated attempts to germinate cultures of the two oldest collections of *Enteridium Rozeanum* (nos. 10 and 11) resulted in the observation of occasional swarm-cells in no. 10, but only very low percentages of germination. Other species gave similar results.

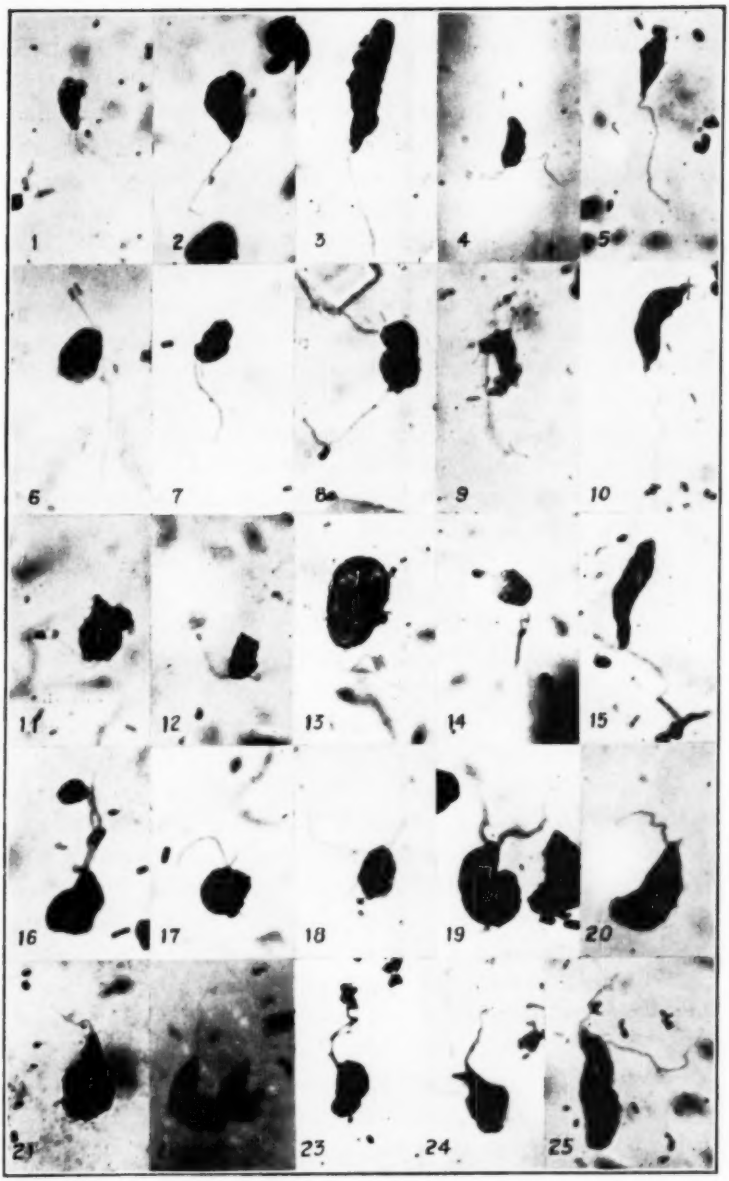
Cultures were made in Syracuse watch glasses using distilled water from which the traces of toxic minerals were removed with powdered charcoal. It was observed that most of the spores placed in the culture dish continued to float on the surface of the water, never becoming wet. This was especially true of those forms having very small spores, such as *Enteridium* and *Lycogala*. Various wetting agents were tried in an attempt to accelerate the wetting and increase the germination of these spores.

Alcohol was the first wetting agent tested. Cayley (**9**) used 20% alcohol for wetting spores of *Didymium* sp., securing approximately 50% germination whether the spores were in the alcohol "a few minutes" or a full hour. Also she used a solution of 0.2% mercuric chloride in a mixture of equal parts of 95% alcohol and water, as a combined wetting agent and bactericide. The resulting cultures were not bacteria-free and germination was poor.

In the tests here reported a number of different dilutions of alcohol were tried, 95% being the strongest and 20% the weakest concentration used. Lower concentrations had negligible wetting effect. After wetting, spores were washed three times with centrifuging and cultured as before. No germination was obtained



Drawings of Figs. 1–25.



FIGS. 1–25. Swarm cells of Myxomycetes.

from spores wetted with alcohol, even in *Reticularia Lycoperdon*, in which nearly 100% germination had been secured without the use of a wetting agent.

Trisodium phosphate was tried next. By experimentation it was found that spores of *Enteridium Rozeanum*, which has the smallest spores of any species used up to the time of these tests, would sink immediately in a 0.5% solution and slowly in a 0.2% solution. In preparing cultures using trisodium phosphate as a detergent, washing was done as when alcohol was the wetting agent. Cultures of *E. Rozeanum* (no. 11), prepared with the use of trisodium phosphate in either 0.5% or 0.2% solution, germinated nearly 100% within one hour. Swarm-cells had not been seen in cultures of this collection before.

Trisodium phosphate in 0.5% solution was used as a detergent in preparation of cultures of a number of other collections. Abundant germination was produced occasionally in *Lycogala epidendrum* (no. 14), but no consistent germination was secured with any species except *E. Rozeanum;* hence toxic effects were suspected.

In the search for an active detergent without toxic effects, two commercial detergents, "Soilax" and "Dreft," were tried, both in 0.5% solution. Both acted as effective wetting agents, but many spores were caught in the foam on the "Dreft" solution and could not be reclaimed by centrifuging. Germination in cultures thus prepared was similar to, and no more satisfactory than, that obtained with trisodium phosphate.

The fact that bile salts greatly lower the surface tension of solutions, as exemplified in the Hay test for bile in urine (**23**), inspired the testing of solutions of sodium glycocholate and sodium taurocholate as wetting agents for preparation of cultures. It was found that either the glycocholate or the taurocholate will wet the spores satisfactorily in 1% solution, but lower concentrations act so slowly as to be inadequate. In equal concentrations, these two salts, or a mixture of them, are equally effective as wetting agents.

Spores of many species of Myxomycetes which did not germinate at all when prepared directly in water or with other detergents, germinated when previously wetted with either of the bile salts. And all those collections which germinated directly in water germi-

nated more quickly and in greater percentage when previously wetted with the bile salts. Of course, the spores of two different collections of the same species frequently show greatly different percentages of germination. When the bile salts are used, other conditions being equal, the differences are substantially reduced.

Germination of spores directly in 1% solution of the bile salts was tried. Protoplasts emerged from the spore cases, but developed no further. However a technique was developed in which only one washing with water is necessary to free wetted spores of the detergent. Approximately 1 cc. of the wetting agent is placed in a centrifuge tube and the spores to be cultured are added and stirred until wetted, a process which usually takes one-half minute. Then the solution is diluted to 5 or 6 cc. with water and promptly centrifuged. The spores are then washed once with distilled water by centrifuging, and are finally cultured in distilled water prepared with charcoal as described above.

The wetting efficiency and the low toxicity of the "Aerosols" recommend them for the purpose of preparing cultures of myxomycete spores. It was hoped that the toxicity of an aerosol solution would be negligible and that a dilution might be found which would be effective in wetting and of such a nature that germination could take place directly in the detergent. As before, spores of *Enteridium Rozeanum* were used as the test organism, and it was found that considerable wetting effect was obtained in solutions as weak as 0.1 ppm. Germination was obtained in solutions as strong as 1000 ppm., the highest percentage of germination in these tests being at 1 ppm. However, when *Reticularia Lycoperdon* was tested in the same dilutions, no germination was obtained in solutions stronger than 1 ppm., and very few swarm-cells were found in solutions stronger than 0.1 ppm. No germination was obtained in aerosol solutions with any of several other forms which were tried, though germination was obtained with all of them in cultures prepared at the same time using the bile salts as described above. Many protoplasts, escaped from the spore cases, were observed to disintegrate explosively in solutions stronger than 1 ppm. in all cultures except those of *Enteridium Rozeanum*. However, germination was obtained in the two forms mentioned above, and the

swarm-cells remained alive in the flagellated form for much longer than was ever observed in cultures prepared otherwise. It is concluded, therefore, that the chemical toxicity of the aerosols is not an important factor, but that the surface tension of the culture solution is of considerable importance. Probably a solution of aerosol could have been used in place of the bile salts solution, with the same washing technique. But since washing would obviate the only advantage of using aerosol, the bile salts were continued, for the sake of uniformity of results.

Alternate wetting and drying, tried on several cultures, gave no noticeable improvement in any case except that of *Physarum flavicomum*, the oldest collection germinated. This culture, prepared by the usual method, germinated in very low numbers. Dried overnight and rewet, it germinated in very high percentage in about six hours.

It was found that in order to preserve the flagella of swarm-cells for observation, the killing agent used in preparation of material for microscopic examination must act very quickly. Smears prepared by air-drying, or even by drying as quickly as possible over mild heat, as is done in the preparation of bacterial mounts, showed recognizable swarm-cells, all of which, however, completely lacked flagella.

The most satisfactory results for temporary mounts were obtained by killing and staining on the slide with a drop of iodine-potassium iodide solution. For this purpose Gram's iodine is used without dilution, density of staining being controlled by varying the proportions of culture solution and iodine solution which are mixed on the slide. Whatever proportions are used, mixing must be accomplished quickly and thoroughly if the flagella are to be preserved for observation.

Permanent slides were prepared for observation of flagella by use of a modification of the Loeffler stain for bacterial flagella (**6**). The mordant and stain were prepared as directed, but the times of application of both mordant and stain were reduced from five minutes to one-half minute. Swarm-cells thus stained with carbol fuchsin were destained to transparency with acid alcohol, counter-

stained with fast green in clove oil and mounted in clarite. On a properly prepared slide the cell body is greenish, the nucleus red, and the flagella deep green to purplish.

Smears were prepared by various means. Slides were thinly coated with albumin fixative, on which a drop of culture solution was placed and inverted over osmic acid fumes. This was then allowed to dry in air. Other smears, killed over osmic acid, were fixed by heating gently after drying. Still others were killed with iodine, as was done in the preparation of temporary mounts, and allowed to stand until the iodine had sublimed. These slides were fixed over heat. The crystals of potassium iodide were dissolved off in distilled water before staining. Equally satisfactory results were obtained with all these methods. The iodine method, being the simplest, was used.

RESULTS AND DISCUSSION

Germination. Cultures were prepared, by the method described above, of 90 collections of Myxomycetes. All orders, all families except the Dianemaceae and Collodermataceae, 30 genera, and 59 species were represented. Germination was obtained in 80 of the collections. At least one culture of each species except *Echinostelium minutum* produced swarm-cells. Following is a list of collections in which swarm-cells were not observed:

21.	*Cribraria intricata*	1940
40–45.	*Echinostelium minutum*	1936–1947
61.	*Stemonitis axifera*	1946
69.	*Craterium leucocephalum*	1941
87.	*Didymium squamulosum*	1947

E. minutum is such a small form that no collection provided sufficient material to be handled as much as is required for wetting by the bile salts method. Cultures had to be prepared directly in water, in hanging drop mounts. It may be that these spores never were adequately wetted, or it may be that fewness of spores resulted in a lack of the mass-action factor postulated by Wilson and Cadman (**59**) in the case of *Reticularia Lycoperdon.*

It has long been recognized that each collection, rather than

each species, is a problem in itself. The bile salts technique for the wetting of spores solved many problems in the way of germination, favorable results being achieved in most cases. But advance wetting before culturing could not be expected to overcome all the "internal factors" referred to in the literature, and so failure was met in the four collections listed above, others of the same species germinating well.

That wetting of spores is an important factor in germination *in vitro* is obvious; for many collections which would not germinate at all when cultured directly in water germinated readily when previously wetted, and forms which germinated in very low numbers when cultured directly in water produced a higher percentage of swarm-cells when wetted before culturing.

Age of the collection seems to have no important bearing on germination within the limits of these experiments. In general, a higher percentage of germination was obtained with material less than a year old than with older material of the same species. However, Gilbert's observation that spores of *Lycogala epidendrum* do not germinate until the fructification is a month old was confirmed. Collection no. 15 was cultured on the day after collection, and each week thereafter, each culture being maintained for one week. No swarm-cells were observed until the fifth culture, made four weeks after collection, which germinated abundantly in about six hours.

Smith germinated spores of collections as much as 32 years old, finding no correlation between age and percentage of germination. In this study were included five collections more than 32 years old:

9.	*Enteridium Rozeanum*	1893
20.	*Cribraria intricata*	1894
24.	*Lindbladia effusa*	1910
58.	*Stemonitis axifera*	1894
78.	*Physarum flavicomum*	1886

Again, no correlation was found between age and percentage of germination. The oldest collection was cultured simultaneously with 20 other collections of 19 different species, the ages ranging from a few months to about 10 years. Swarm-cells were observed in *Physarum flavicomum* before half of the others germinated. As

is mentioned above, germination in this very old collection was markedly improved by drying and rewetting the culture. None of the other 20 collections responded to this treatment, and so within six hours after rewetting the percentage of germination in *P. flavicomum* was much higher than was ever found in any of the rest. The *Stemonitis axifera* was cultured with three others of the same species, ranging from about one year to 13 years. The freshest culture germinated 24 hours ahead of the rest, but percentage of germination was about equal in all four, and apparent vigor of the swarm-cells was equal in all cultures.

Seventeen species not listed as previously germinated were germinated in these tests. Two monotypic genera are represented: *Clastoderma Debaryanum* and *Cienkowskia reticulata*. Kambly (**34**) reports that attempts to produce plasmodia of *C. Debaryanum* from spores were unsuccessful, but it is not clear whether he observed germination of spores and production of swarm-cells of this species. He further states that the plasmodium of *C. Debaryanum* would not grow in artificial media away from its natural substratum. No special care was given the spores in these tests, but swarm-cells were produced in abundance.

Emergence of protoplasts from spore cases was observed in every culture of every form in which germination occurred. In no case was a flagellum observed on a protoplast at the time of emergence. In these cultures, flagella were formed only after a resting period of varying duration, and in several species smears made at frequent intervals after emergence was first observed, showed the growth of the flagellum to take place at a rather slow rate, requiring as much as two hours in some cultures.

In no culture was the emergence of more than one protoplast from a single spore observed. All of the swarm-cells of *Trichia affinis* and of *Physarum mellcum*, and most of those of *Trichia favoginea*, which were observed, were smaller than would be expected if a single swarm-cell were produced by each spore. But the protoplasts seen emerging were the full content of the spore, and so it is assumed that the size of the swarm-cells is the result of divisions outside the spore case.

No germination was obtained at any time if the room tempera-

ture was above 32° or 33° C. For this reason, experimental work had to be discontinued during the hotter days of the summer. Also, it was observed that whenever germination did take place near the upper limit of tolerance, more myxamoebae and fewer swarm-cells were found in the cultures than when cultures of the same collections germinated at temperatures nearer 25° C.

As is stated above, it is believed that the surface tension of the culture solution is of considerable importance in the germination of myxomycete spores. Unfavorable response to the low surface tension is the only obvious explanation of the reaction to aerosol solutions observed. The fact that no germination, even of *Enteridium Rozeanum*, was obtained in the bile salts solution without washing may be interpreted as implying that the bile salts are toxic, whether the surface tension of the solution is unfavorable or not. All observers report best germination in neutral or slightly acid media. The apparent toxicity of trisodium phosphate and the commercial detergents is probably due to their alkalinity.

Every culture prepared was observed to be contaminated with bacteria from the time of preparation, and with various protozoa if maintained more than 24 hours. Ciliates of the genus *Colpoda* and a flagellate identified as *Cercobodo agilis* were the most common. The flagellate is of approximately the same size as a myxomycete swarm-cell, but moves with a gliding motion so different from a swarm-cell that the two forms are not easily confused even under the lowest powers of the microscope. *Cercobodo agilis* has one anterior flagellum and a second flagellum equal to the first arising near the middle of the body. The nucleus is approximately central, and the second flagellum arises near the nucleus. This flagellum always trails and appears to be posterior, but that it is not attached posteriorly is easily seen on living mounts as well as on permanent, stained slides if high magnification is used. The bipolar flagellate frequently reported in the literature was not seen in any culture. The flagellate here described occurred in nearly every culture, was most numerous in cultures of *Ceratiomyxa,* and flourished and encysted prior to observation of swarm-cells in all cultures of all collections more than 20 years old.

Flagellation. Some biflagellate swarm-cells were observed in

every species studied. In several species the proportion of swarm-cells obviously biflagellate was nearly 100%. *Lycogala epidendrum*, *Cribraria purpurea* (FIG. 3), *Oligonema Schweinitzii* (FIG. 7), *Leocarpus fragilis* (FIG. 18), *Fuligo septica* (FIG. 17), and *Physarella oblonga* (FIGS. 19, 20) were the most notable among these. In most species the second flagellum is usually difficult to see, but with careful, experienced observation it can be found on nearly every swarm-cell studied. Zygotes with four flagella, representing two biflagellate swarm-cells fused posteriorly, were found occasionally in *Dictydiaethalium plumbeum*, *Cribraria purpurea*, *Oligonema Schweinitzii* (FIG. 8), *Stemonitis fusca*, and *Tubifera ferruginosa*.

On a great majority of swarm-cells of most species, the second flagellum is very short, scarcely more than 1 μ long. (Swarm-cells in this group will be referred to as "type I.") See figures 1, 4, 5, and 10. Other swarm-cells have the second flagellum somewhat longer, measuring approximately half the length of the longer flagellum (type II), as shown in figures 12, 14, 15, and 18. And on a few individuals the two flagella are nearly equal (type III), as shown in figures 3, 20, 23, and 25. Previous accounts, though maintaining the assumption that swarm-cells of Myxomycetes are characteristically uniflagellate, have brought out this variation in the length of the second flagellum. E. C. Smith (**54**, **55**) published photomicrographs showing biflagellate swarm-cells of *Reticularia Lycoperdon*, *Dictydiaethalium plumbeum*, *Lepidoderma tigrinum*, and *Physarum cinereum* with the second flagellum distinct, and *Badhamia orbiculata* in which only a dark line on the body of the cell is seen. All of the swarm-cells in his pictures are type I. Gilbert (**16**) showed *Stemonitis fusca* with two flagella nearly equal, though the rest of the forms he drew were definitely heterocont. Ellison (**15**) drew swarm-cells of *Stemonitis fusca* and *S. axifera* with two flagella nearly equal even though the others he records have one flagellum much shorter than the other. (*Cf.* FIGS. 14, 15, and 16.)

In a previous report on this same investigation (**14**) it was stated that all swarm-cells of *Stemonitis splendens* are of type III,

but all other species studied up to that time have swarm-cells of type I. It was further stated that the ratio of the lengths of the two flagella is quite constant in any given species. Further study has revealed exceptions to these generalizations. Some smears of *Stemonitis splendens* have contained numerous swarm-cells with type II flagellation. None of type I, however, have been found in this species. More variation in the length of the second flagellum is found in those species in which it is usually of type II or type III than in those in which it is shorter. However, smears of *Physarella oblonga* contain about 10% swarm-cells of type III, the rest being of type I. None of type II was found. (*Cf.* FIGS. 19 and 20.)

All swarm-cells of species of the Reticulariaceae and of the Trichiaceae which were studied were of type I, and all swarm-cells of *Tubifera ferruginosa*, the only member of the Liceaceae included in this investigation, were also of type I. In the Physaraceae most of the swarm-cells of the larger genera are of type I, and most of the swarm-cells of the remaining genera are of the longer types; but there is some variation in most species. Most members of the Cribrariaceae, Stemonitaceae, and Didymiaceae have swarm-cells predominantly of types II and III, though here again, there is considerable variation within most species.

The second flagellum, in addition to being very short, is usually recurved so as to be almost indistinguishable from the outline of the cell itself (FIG. 21). Even in those species in which both flagella are relatively long, one is usually trailing, so that it is easily overlooked (FIG. 6). The significance of this point is borne out in the following observation: A temporary mount was prepared from a culture of swarm-cells of *Dictydiaethalium plumbeum*. The mount was killed and stained with Gram's iodine. Using the 90 × oil immersion objective, the microscope was focused upon a swarm-cell in which both flagella were easily seen. Then, by touching the edge of the cover slip with a dissecting needle, the mount was disturbed while the original swarm-cell was kept in view. This swarm-cell was observed to roll over and again come to rest. In its new position, the shorter flagellum could not be seen with any manipulation of the microscope. A second swarm-cell which pre-

sented only the longer flagellum to view was found, and by similarly disturbing the cover slip the swarm-cell was maneuvered until the shorter flagellum could also be seen. This same demonstration of the fact that the shorter flagellum may be—and frequently is—hidden by the body of the cell, was also performed on mounts of *Fuligo septica* and *Arcyria denudata*.

The second flagellum is clearly visible on only a very few swarm-cells in most mounts. Some mounts stained with Loeffler's technique were destained to transparency with acid alcohol. On swarm-cells thus prepared the flagella remained clearly stained, but the body of the cell was sufficiently destained so that the nucleus and other cellular details were visible. When swarm-cells are properly stained by this method, careful focusing on the anterior portion of the body of the cell will reveal a dark line reaching backward from the apex to the base of the conical anterior portion. Occasionally this dark line will be found lying across the conical portion, either on or underneath the body of the cell, but most frequently it is barely distinguishable from the outline of the cell. It is not found on those swarm-cells on which the second flagellum is clearly visible apart from the body of the cell. It is believed that this is the shorter flagellum which ordinarily is closely appressed to the anterior portion of the cell. Figures 5, 10, and 21 illustrate this condition to some extent, but the cases in which the second flagellum is most difficult to see could not, of course, be photographed.

CONCLUSIONS

Spores of 80 collections representing 58 species of Myxomycetes were germinated. Germination was induced or improved by previous wetting, using a solution of the bile salts as a wetting agent. The flagellation of the swarm-cells of these species was observed in temporary and permanent preparations. In some species it was possible to see two flagella on nearly every swarm-cell observed. In all other species, two flagella were clearly visible on some swarm-cells, and it was demonstrated by manipulation of fluid mounts that the second flagellum could be brought into view even though not originally visible. From this it is inferred that if ade-

quate technique were used, the second flagellum would be found on all myxomycete swarm-cells.

Most of the biflagellate swarm-cells observed in this study were definitely heterocont. In most species the two flagella differ greatly in length. Even in those swarm-cells on which the two flagella are nearly equal, there is usually a distinct, though slight, difference in length.

Groupings of species based on the type of flagellation usually found on swarm-cells of each species show little correlation with the currently accepted classification of the Myxomycetes based on morphology of the fructifications. This, and the fact that there is considerable variation in the length of the second flagellum within some species, seems to make the taxonomic significance of the second flagellum, within the Myxomycetes, doubtful.

The Myxomycetes and the Plasmodiophorales have long been considered related groups by many investigators. Formerly, the reported existence of anteriorly uniflagellate zoospores in the reproductive cycle of both groups was regarded as strong evidence of this relationship. However, Ledingham (**36**, **37**, **38**) showed that the zoospores of the Plasmodiophorales are anteriorly biflagellate, the second flagellum being very short and hence easily obscured. As a result of Ledingham's finding, the supposed difference in flagellation was thought by many to emphasize a separation between the two groups.

The existence of two blepharoplasts has been reported in several species of Myxomycetes (**16**, **22**, **56**). Bessey (**5**) regards the second blepharoplast as a vestige of the biflagellate condition, indicating that the Myxomycetes and the Plasmodiophorales have arisen from a common ancestor. He considers the loss of the second flagellum as evidence that the Myxomycetes are of higher phylogenetic position.

Insofar as flagellation is of phylogenetic significance, the existence of the second flagellum in the swarm-cells of Myxomycetes, as demonstrated by this study, may indicate a closer relationship with the Plasmodiophorales than has recently been supposed.

This work was done in the Mycological Laboratory of the State University of Iowa under the direction of Professor G. W. Martin.

APPENDIX

CHECK LIST OF MYXOMYCETES PREVIOUSLY GERMINATED

In each instance in which the currently accepted name of a species differs from that used by an author cited, the synonym is given, indented in the first column, opposite the name of the author.

Species	Worker	References
Amaurochaete fuliginosa (Sow.) Macbr.		
as *A. atra*	Lister	39, 40
	Constantineanu	11
as *A. atra*	Jahn	29
as *A. atra*	Schunemann	48
Arcyria cinerea (Bull.) Pers.	F. A. Gilbert	21
	Smart	52, 53
as *A. digitata*	Smart	52, 53
Arcyria denudata (L.) Wettst.		
as *A. punicea*	deBary	3
	F. A. Gilbert	19, 20, 21
	Smart	52, 53
Arcyria ferruginea Sauter	Pinoy	45
Arcyria incarnata Pers.	Hoffmann	25
	Constantineanu	11
	F. A. Gilbert	19, 20, 21
	Smart	52, 53
Arcyria nutans (Bull.) Grev.	F. A. Gilbert	21
	Smart	52, 53
Arcyria Oerstedtii Rost.	F. A. Gilbert	20, 21
	Smart	52, 53
Arcyria pomiformis (Leers) Rost.	F. A. Gilbert	21
	Smart	52, 53
Badhamia affinis Rost.	Smith	54
Badhamia capsulifera (Bull.) Berk.		
as *B. hyalina*	McClatchie	43
Badhamia Curtisii (Berk.) Rost.		
as *B. rubiginosa*	F. A. Gilbert	21
as *B. rubiginosa*	Smith	54
as *B. rubiginosa*	Smart	52, 53
as *Physarum rubiginosum*	Smart	52, 53
Badhamia lilacina (Fries) Rost.	F. A. Gilbert	19, 20, 21
	Smith	54
	Smart	52, 53
Badhamia macrocarpa (Ces.) Rost.		
as *Physarum macrocarpum*	Hoffmann	3
	Constantineanu	11
	Jahn	29
Badhamia ovispora Racib.	Smart	52, 53
Badhamia panicea (Fries) Rost.	Smith	54, 55

Species	Worker	References
Badhamia utricularis (Bull.) Berk.	Lister	39
as *B. magna*	F. A. Gilbert	19, 20, 21
	Smith	54, 55
as *B. magna*	Smith	54
	Smart	52, 53
as *B. magna*	Smart	52, 53
Ceratiomyxa fruticulosa (Muell.) Macbr.		
as *Ceratium* spp.	Famintzin & Woronin	41
	Olive	44
	Jahn	32
	Sinoto & Yuasa	49
	H. C. Gilbert	22
	Smart	52, 53
Comatricha elegans (Racib.) Lister	Smart	52, 53
Comatricha irregularis Rex	F. A. Gilbert	21
Comatricha laxa Rost.	Smart	52, 53
Comatricha longa Peck		
as *C. longa* var. *flaccida* Minakata	Sinoto & Yuasa	49
Comatricha nigra (Pers.) Schroet.		
as *Stemonitis obtusata*	deBary	3
	Smart	52, 53
Comatricha pulchella (Bab.) Rost.	Smart	52, 53
Comatricha typhoides (Bull.) Rost.	F. A. Gilbert	19, 21
Cornuvia Serpula Rost.		
as *Arcyria anomala*	deBary	3
Craterium leucocephalum (Pers.) Ditm.	Smith	54
	Smart	52, 53
Cribraria aurantiaca Schrad.		
as *C. vulgaris* var. *aurantiaca*	Constantineanu	11
	Smart	52, 53
Cribraria elegans Berk. & Curt.	Smart	52, 53
Cribraria intricata Schrad.	Smart	52, 53
Cribraria minutissima Schw.	Smart	52, 53
Cribraria tenella Schrad.	F. A. Gilbert	21
	Smart	52, 53
Diachaea leucopodia (Bull.) Rost.	McClatchie	43
	Smith	55
	Smart	52, 53
Dictydiaethalium plumbeum (Schum.) Rost.	F. A. Gilbert	17, 19, 20, 21
	Smith	55
	Smart	52, 53
Dictydium cancellatum (Batsch) Macbr.	Constantineanu	11
	F. A. Gilbert	20
as *D. cancellatum* var. *purpureum*	Sinoto & Yuasa	49
	Smart	52, 53
as *D. cancellatum* var. *purpureum*	Smart	52, 53
Diderma effusum (Schw.) Morgan	Smith	54

Species	Worker	References
Diderma globosum Pers.	Smart	52, 53
Diderma radiatum (L.) Morgan	Smith	54
Diderma testaceum (Schrad.) Pers.	Smart	52, 53
Didymium difforme (Pers.) S. F. Gray		
as *D. Libertianum*	deBary	3
as *Physarum album*	Cienkowski	10
	Lister	40
as *D. difforme* var. *comatum*	Lister	40
	Schunemann	48
	Jahn	29
	Skupienski	50, 51
	Cayley	9
Didymium melanospermum (Pers.) Macbr.	F. A. Gilbert	21
	Smith	54
	Schunemann	48
	Smart	52, 53
Didymium nigripes (Link) Fries	Pinoy	47
	Heitzmanowna	24
	Schunemann	48
	Cadman	7
	Abe	33
	von Stosch	56
	Kambly	34
Didymium Serpula Fries	Cienkowski	10
Didymium squamulosum (Alb. & Schw.) Fries		
as *Physarum Tussilaginis*	Kent	39
as *Didymium praecox*	deBary	3
as *Didymium effusum*	Pinoy	45, 46
	Constantineanu	11
as *Didymium effusum*	Jahn	30
	F. A. Gilbert	21
	Smith	54, 55
	Schunemann	48
	Smart	52, 53
	Kambly	34
Didymium xanthopus (Ditm.) Fries		
as *D. nigripes* var. *xanthopus*	F. A. Gilbert	19, 20, 21
as *D. nigripes* var. *xanthopus*	Cayley	9
	von Stosch	56
as *D. nigripes* var. *xanthopus*	Smart	52, 53
	Kambly	34
Enerthenema papillatum (Pers.) Rost.	Smith	54
Enteridium olivaceum Ehrenb.	Smith	55
Enteridium Rozeanum (Rost.) Wing.	Durand	13
	Jahn	29
	F. A. Gilbert	16, 19, 20, 21
	Smart	52, 53
Erionema aureum Penzig	Abe	33

Species	Worker	References
Fuligo septica (L.) Wiggers		
as *Aethalium septicum*	deBary	3
	McClatchie	43
as *Aethalium septicum*	Constantineanu	11
	F. A. Gilbert	18, 19, 20
	Cook & Holt	12
	Smith	55
	Abe	33
	Yuasa	60
	Smart	52, 53
	Kambly	34
	Ellison	15
Hemitrichia clavata (Pers.) Rost.	F. A. Gilbert	16, 19, 21
	Smith	54, 55
	Smart	52, 53
Hemitrichia Serpula (Scop.) Rost.	F. A. Gilbert	21
	Smith	54
	Smart	52, 53
Hemitrichia stipata (Schw.) Macbr.		
as *Arcyria stipata*	F. A. Gilbert	20, 21
Hemitrichia Vesparium (Batsch) Macbr.		
as *Trichia rubiformis*	deBary	1, 3
as *Trichia pyriformis*	deBary	3
as *Hemiarcyria rubiformis*	McClatchie	43
	F. A. Gilbert	19, 20, 21
	Smith	54
	Smart	52, 53
Lamproderma arcyriodes (Somm.) Rost.		
as *L. violaceum*	Smith	54, 55
Lamproderma arcyrionema Rost.	Smart	52, 53
Lamproderma columbinum (Pers.) Rost.	F. A. Gilbert	21
Leocarpus fragilis (Dicks.) Rost.		
as *L. vernicosus*	Hoffmann	25
	Constantineanu	11
	F. A. Gilbert	18, 19
	Schunemann	48
	Smart	52, 53
Lepidoderma tigrinum (Schrad.) Rost.	Smith	54, 55
Lindbladia effusa (Ehrenb.) Rost.	Jahn	30
Lycogala epidendrum (L.) Fries	deBary	3
as *L. miniatum*	Constantineanu	11
	F. A. Gilbert	19, 20, 21
	Cook & Holt	12
	Smart	52, 53
Lycogala flavo-fuscum (Ehrenb.) Rost.	F. A. Gilbert	20, 21
	Smith	54
as *L. flavo-fuscum* var. *solida*	Smith	54
	Smart	52, 53

Species	Worker	References
Mucilago spongiosa (Leysser) Morgan	F. A. Gilbert	18
Oligonema flavidum (Peck) Peck	Smart	52, 53
Perichaena corticalis (Batsch) Rost.		
as *Licea pannorum*	deBary	3
as *Licea pannorum*	Cienkowski	10
Perichaena depressa Libert	Constantineanu	11
	Smart	52, 53
Physarella oblonga (Berk. & Curt.) Morgan	Sinoto & Yuasa	49
	Smart	52, 53
Physarum albescens Macbr.		
as *P. fulvum*	Smith	54
Physarum bivalve Pers.		
as *P. sinuosum*	F. A. Gilbert	21
	Smart	52, 53
Physarum cinereum (Batsch) Pers.	F. A. Gilbert	21
	Smith	54, 55
	Smart	52, 53
Physarum compressum Alb. & Schw.	F. A. Gilbert	18, 21
	Kambly	33
Physarum crateriforme Petch	Abe	33
Physarum didermoides (Pers.) Rost.	Constantineanu	11
	Jahn	30
as *P. lividum* List.	Smith	54
	Smart	52, 53
	Kambly	34
Physarum digitatum G. Lister & Farquh.	Smart	52, 53
Physarum flavicomum Berk.	Smart	52, 53
Physarum globuliferum (Bull.) Pers.	Smart	52, 53
Physarum leucophaeum Fries	Smart	52, 53
Physarum leucopus Link		
as *Didymium leucopus*	Cienkowski	3
	Schunemann	48
	F. A. Gilbert	18, 21
	Smart	52, 53
Physarum notabile Macbr.		
as *P. connatum*	F. A. Gilbert	18, 21
	Smith	54
Physarum nucleatum Rex	Smart	52, 53
Physarum nutans Pers.		
as *P. albipes*	deBary	3
	Smart	52, 53
Physarum polycephalum Schw.	Howard	26
	Smart	52, 53
Physarum pulcherrimum Berk. & Rav.	Smart	52, 53
Physarum Serpula Morgan	F. A. Gilbert	18, 21
	Smith	54
	Smart	52, 53
Physarum straminipes Lister	Smith	55

Species	Worker	References
Physarum virescens Ditmar	F. A. Gilbert	18, 21
	Smith	54
Physarum viride (Bull.) Pers.	F. A. Gilbert	19, 21
	Smart	52, 53
Reticularia Lycoperdon Bull.		
as *R. umbrina*	deBary	3
as *R. umbrina*	McClatchie	43
	Jahn	29
	F. A. Gilbert	18, 19, 21
	Cook & Holt	12
	Wilson & Cadman	59
	Smith	55
	Schunemann	48
	Smart	52, 53
	Kambly	33
Stemonitis axifera (Bull.) Macbr.		
as *S. ferruginea*	Jahn	29
as *S. ferruginea*	F. A. Gilbert	19, 21
as *S. ferruginea*	Smith	55
	Smart	52, 53
as *S. ferruginea*	Ellison	15
Stemonitis flavogenita Jahn	Smith	54, 55
Stemonitis fusca Roth.	Hoffmann	25
	deBary	3
	Constantineanu	11
	F. A. Gilbert	16, 19, 21
as *S. fusca* var. *rufescens*	F. A. Gilbert	21
	Smith	54
	Abe	33
	Smart	52, 53
	Ellison	15
Stemonitis splendens Rost.		
as *S. splendens* var. *flaccida*	Constantineanu	11
as *S. flaccida*	Jahn	28, 29
	F. A. Gilbert	19
as *S. splendens* var. *flaccida*	F. A. Gilbert	19, 21
as *S. splendens* var. *flaccida*	Cook & Holt	12
	Smith	54
as *S. splendens* var. *flaccida*	Sinoto & Yuasa	49
	Smart	52, 53
as *S. splendens* var. *flaccida*	Smart	52, 53
Trichia Botrytis (J. F. Gmel.) Pers.		
as *T. fragilis*	Lister	39
	Smith	54, 55
Trichia contorta (Ditm.) Rost.	F. A. Gilbert	21
	Smart	52, 53

Species	Worker	References
Trichia favoginea (Batsch) Pers.	Smith	54, 55
	Smart	52, 53
Trichia floriformis (Schw.) G. Lister as *T. lateritia*	F. A. Gilbert	19, 21
	Smith	55
	Smart	52, 53
Trichia persimilis Karst.	F. A. Gilbert	21
	Smith	54
	Smart	52, 53
Trichia pusilla (Hedw.) Martin as *T. fallax*	Lister	39
Trichia scabra Rost.	F. A. Gilbert	21
	Smith	55
Trichia varia (Pers.) Pers.	deBary	4
	F. A. Gilbert	21
	Cook & Holt	12
	Smart	52, 53
Tubifera ferruginosa (Batsch) J. F. Gmel. as *Tubulina cylindrica*	Jahn	30

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EXPLANATION OF FIGURES

Photomicrographs and Explanatory Drawings of Myxomycete Swarmcells. FIG. 1. *Ceratiomyxa fruticulosa*. FIG. 2. *Tubifera ferruginosa*. FIG. 3. *Cribraria purpurea*. FIG. 4. *Dictydium cancellatum*. FIG. 5. *Enteridium Rozeanum*. FIG. 6. *Arcyria cinerea*. FIG. 7. *Oligonema Schweinitzii*. FIG. 8. Zygote of *Oligonema Schweinitzii*. FIG. 9. *Trichia affinis*. FIG. 10. *Trichia favoginea*. FIG. 11. *Lamproderma arcyrionema*. FIG. 12. *Lamproderma Sauteri*. FIG. 13. *Lamproderma scintillans*. FIG. 14. *Stemonitis axifera*. FIG. 15. *Stemonitis fusca*. FIG. 16. *Stemonitis splendens*. FIG. 17. *Fuligo septica*. FIG. 18. *Leocarpus fragilis*. FIG. 19. *Physarella oblonga*. FIG. 20. *Physarella oblonga*. FIG. 21. *Physarum didermoides*. FIG. 22. *Physarum flaviconium*. FIG. 23. *Physarum melleum*. FIG. 24. *Physarum viride*. FIG. 25. *Didymium xanthopus*. All photographs and drawings are × 1000.

A NEW AQUATIC SPECIES OF PYTHIUM *

ADRIAN W. POITRAS

(WITH 17 FIGURES)

In the course of a study of the aquatic Phycomycetes of Illinois which has been in progress during the last several years, eighty-five isolates representing the genus *Pythium* have been obtained. It was found that these represented sixteen different species, including one whose characteristics are such that it is being proposed as new in the sub-genus Sphaerosporangium.

The described species so far recognized in Illinois are as follows: *Pythium monospermum* Pringsheim, *P. dissotocum* Drechsler, *P. gracile* Schenk, *P. tenue* Gobi, *P. catenulatum* Matthews, *P. torulosum* Coker and Patterson, *P. inflatum* Matthews, *P. aphanidermatum* (Edson) Fitzpatrick, *P. pulchrum* von Minden, *P. ultimum* Trow, *P. splendens* Braun, *P. vexans* de Bary, *P. complectans* Braun, *P. debaryanum* Hesse, *P. irregulare* Buisman.

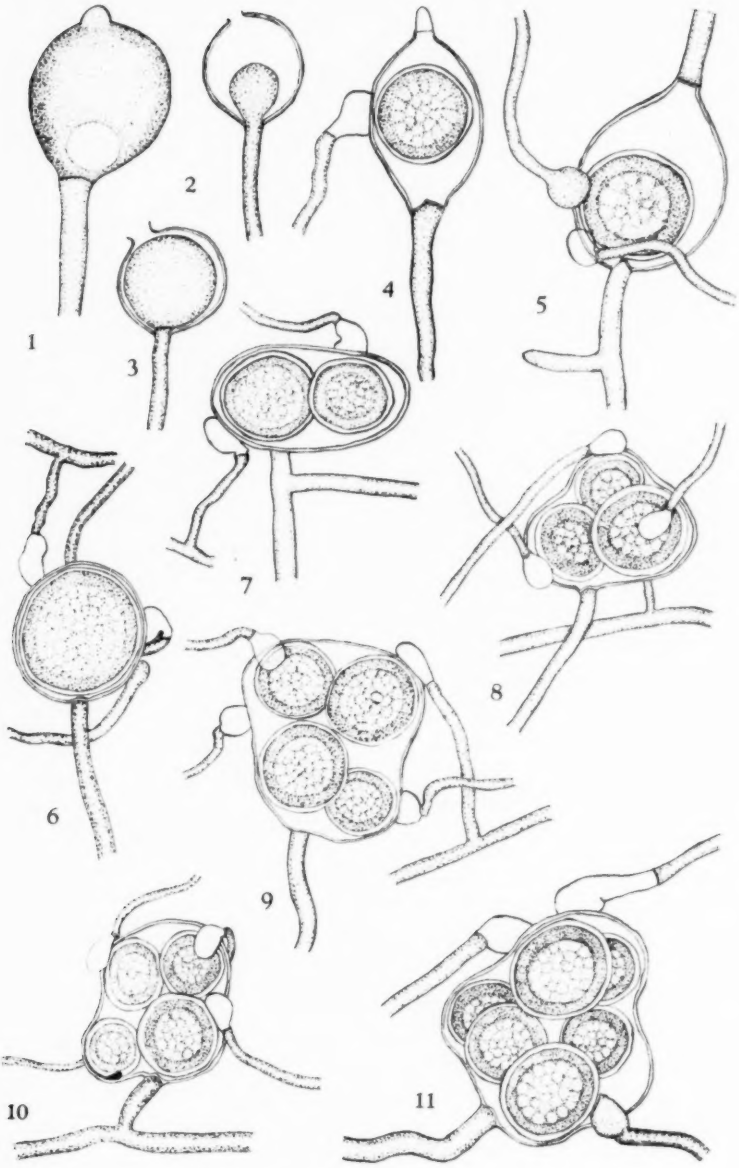
The following new species was found:

Pythium multisporum sp. nov.**

Mycelio ramosiore, 3.0–8.4 μ diam. Sporangiis sphaericis vel subsphaericis, terminalibus; 29.5–55.0 μ diam.; proliferentibus; zoosporis 30–50, reniformibus, a latere biflagellatis, quae in vesica sporangio contigua formantur et sunt 8.4–10.5 μ lata, 10.5–16.8 μ longa si in cystibus gignuntur. Oogoniis sphaericis, subsphaericis vel enormibus; levibus; terminalibus vel subterminalibus, quae si singula gignuntur sunt 18.9–39.9 μ diam., si composita, 26.4–48.8 μ lata, 39.6–74.8 μ longa. Oosporis sphaericis vel subsphaericis; 10.5–29.4 μ diam.; apleroticis; quae singulae vel plures singulis oogoniis insunt et cuticulas leves et paulo crassiores habent. Antheridiis androgynis et diclinis, quae singula vel plura singulis oogoniis insunt et quorum pars extrema ad oogonium adhaeret.

* The writer wishes to express his gratitude to Dr. Leland Shanor for his advice and counsel during this study and the writing of this paper. He also wishes to thank Mr. Everett S. Beneke for making available for study isolates of *Pythium* from his collections taken throughout Illinois.

** Latin diagnosis prepared with the assistance of Prof. R. P. Oliver, Department of Classics, University of Illinois.



FIGS. 1–11. *Pythium multisporum.*

Hyphae freely branched and well developed on most culture media, measuring 3.0–8.4 μ in diameter. Sporangia spherical, subspherical, infrequently ellipsoidal or pyriform, occasionally papillate; terminal on lateral branches; measuring 29.5–55.0 μ, average 42.8 μ in diameter; proliferous, the secondary sporangia usually formed within the walls of the primary ones; zoospores 30–50, laterally biflagellate and reniform, formed within a vesicle borne adjacent to the sporangium and measuring 8.4–10.5 $\mu \times$ 10.5–16.8 μ upon encystment. Oogonia spherical, ovoid or irregular, smooth; terminal or intercalary, unisporous oogonia measuring 18.9–39.9 μ, average 30.4 μ in diameter, multisporous oogonia measuring 26.4–48.4 $\mu \times$ 39.6–74.8 μ, size largely dependent upon the number of oospores. Oospores spherical to ovoid, one to several per oogonium, aplerotic, measuring 10.5–29.4 μ, averaging 21.6 μ in diameter, with a smooth, moderately thickened wall. Antheridia androgynous and diclinous, one to several, usually two, per oogonium; the antheridial stalk of variable length; the antheridial cell cut off by a cross-wall, clavate to swollen, closely to moderately applied terminally to the oogonium, measuring 6 to 10 μ by 9 to 15 μ.

Isolated from a soil sample taken from the bank of a branch of the Illinois River at Havana, Illinois, on October 19, 1947.

This fungus grows quite readily on most types of agar media and on boiled hempseed in water. Growth on agar media is limited usually to surface and subsurface mycelia; however, scant aerial hyphae developed on malt agar. The mycelial growth on malt, malt-peptone and potato-dextrose agar media is quite profuse, whereas on corn-meal agar growth is very sparse. All stages are produced readily on hempseed in water and, therefore, most of the material used for this study was grown by this method.

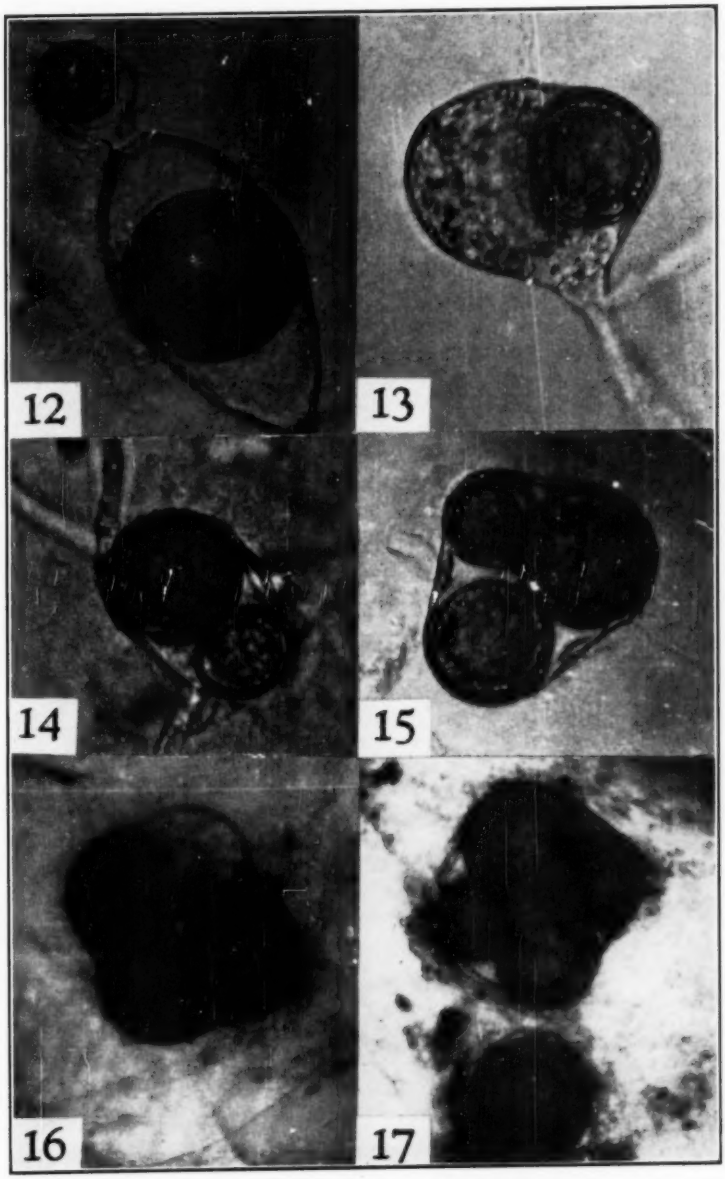
The most distinguishing characteristic of this fungus is that many of its oogonia contain more than one oospore. While about seventy-seven per cent typically contain a single oospore, about seventeen per cent contain two oospores, five per cent contain three oospores and the remaining one per cent contain from four to six oospores. Sorokin (**1872**) described *Pythium polysporum* as a species in which the oogonia contained several oospores, the oogonia measuring 40.0–50.0 μ in diameter and the oospores measuring 7–8 μ in diameter. Butler (**1907**) excludes *P. polysporum* from the genus *Pythium* on the basis of its sexual and asexual stages,

and both Matthews (**1931**) and Middleton (**1943**) concur in this opinion, Matthews suggesting that it might represent a new undiagnosed genus of the Pythiaceae. It is quite evident that our isolate is not the same as the one described by Sorokin.

Oogonia with two oospores are infrequently produced in a limited number of species of *Pythium*. The most closely related of these to *Pythium multisporum* is *Pythium pulchrum*, but *P. pulchrum* differs from our isolate by possessing the following characteristics: catenulate sporangia, the presence of a discharge tube of variable length between the sporangium proper and the vesicle, the lack of internal sporangial proliferation, and hypogynous antheridia.

Internally proliferating sporangia have been reported for several species of *Pythium*, including *P. proliferum*, *P. marsipium*, *P. polytylum*, *P. helicoides*, *P. oedochilum*, *P. palingenes*, and *P. nagii*, but none typically produce more than one oospore in an oogonium. *Pythium proliferum* differs also from *P. multisporum* in that its sporangia and oogonia are smaller in diameter and that hypogynous antheridia are characteristic. *Pythium marsipium* has large spherical or asymmetrically utriform sporangia which are papillate while *P. polytylum* has several reserve globules within the oospores and elongate, cylindrical or irregularly shaped antheridia. *Pythium helicoides* is distinguished from our isolate also by the fact that the antheridial stalk is coiled around the oogonial stalk. Papillate sporangia and antheridia which are wavy, contoured and attached over their entire length serve to separate *P. oedochilum* and *P. palingenes* from *P. multisporum*. *Pythium nagii* is obviously distinguished from our isolate because of the small size of its sexual and asexual structures, the proliferous nature of its sporangia being the only factor serving to unite it with this group. The presence of only a moderately thickened wall and a single reserve globule in the oospore, the fact that oogonia characteristically often possess several oospores, and the fact that the sporangia are regularly spherical to subspherical serve adequately enough to separate *P. multisporum* from other described species.

The pathogenicity of *Pythium multisporum* has not been studied.



FIGS. 12–17. *Pythium multisporum.*

SUMMARY

From soil and water samples taken throughout the State of Illinois during the past several years sixteen species of the genus *Pythium* have been isolated and identified, including a new species belonging to the sub-genus Sphaerosporangium here described and named whose most destinctive characteristic is that, for a species of *Pythium*, a relatively high per cent of the oogonia possess from two to six oospores.

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EXPLANATION OF FIGURES

FIGS. 1, 2, and 3: terminal sporangia, figures 2 and 3 showing proliferation. FIGS. 5, 6, 7, 8, 9, 10 and 11 of oogonia; figures 4, 5, and 6 showing variation in terminal and intercalary single spored oogonia; figure 7, an oogonium with two oospores; figure 8, an oogonium with three oospores; figures 9 and 10, oogonia with four oospores; figure 11, an oogonium with six oospores. (All figures magnified 600 ×.)

FIGS. 12–17. Photomicrographs of oogonia. Figure 12, chain of two oogonia representing extremes in oospore size; figures 13 and 14, oogonia with two oospores, the oospore on the left in figure 13 being aborted; figure 15, an oogonium with three oospores, each oospore being of different size; figures 16 and 17 are oogonia with four oospores, one oospore in figure 16 being aborted. (Figure 12 approximately 800 ×, others 575 ×.)

PRESERVATION OF SAPROLEGNIACEAE BY THE MINERAL OIL METHOD

HELEN SIMPSON REISCHER[1]

The mineral oil method, originally developed for the conservation of bacterial cultures, has been applied to filamentous fungi first by Sherf (**1943**), later by Norris (**1944**) and Wernham (**1946**), and most widely by Buell and Weston (**1947**). The latter remarked upon the peculiar applicability of this simple method, which they used successfully in preserving a collection of 1800 cultures including, as well as higher fungi, Actinomycetes and terrestrial Phycomycetes, to delicate non-sporulating forms.

At the time when Dr. Weston was carrying on this work the author was faced with the problem of maintaining in pure culture a number of aquatic Phycomycetes of the family Saprolegniaceae. These fungi were being kept on Difco corn-meal agar slants in a 4–10 degree Centigrade coldroom. Such slants have a very uncertain life, drying more or less unpredictably in from three to fifteen or nineteen months. Many species of this family produce resistant spores (oospores), for which a successful method of germination has recently been reported (Ziegler, **1948**). These spores have been reported as requiring a resting period, the limits of which are not precisely known, so that cultures derived from oospores would not always be readily available. Oospores do not appear suited to lyophilization. Of twelve isolates of *Achlya* with abundant mature oospores which were lyophilized, using the method of Raper and Alexander (**1945**), none were viable three months later. Microscopic examination revealed disorganization of the oospores. The encysted zoospores (cystospores) of seven isolates of *Achlya* disintegrated under lyophilization. The oospore-producing species then, as well as the various forms of heterothallic

[1] The author wishes to thank Wm. H. Weston, under whose direction this work was begun, and Wm. J. Robbins, in whose laboratory it was continued.

species and those homothallic species which typically produce few if any mature oospores, must be considered for purposes of maintenance as non-sporulating mycelia for which the mineral oil method might be suitable.

As they were obtained, seventy-one isolates, including eight genera, were grown on Difco corn-meal agar slants which were then flooded with mineral oil, following the method of Buell and Weston (**1947**), and subsequently incubated at 4–10 degrees Centigrade. Of these, four isolates (*Achlya* and *Brevilegnia*) were lost in 1946 at 6–11 months. At the same time 11 slants of other isolates (*Achlya*) were discarded—four because they failed

TABLE 1

RETENTION OF VIABILITY BY CULTURES OF SAPROLEGNIACEAE UNDER MINERAL OIL

Genus	Number of different isolates	Age of oldest culture or cultures viable May, 1948
Achlya	5	30 months
	1	24 months
	5	19 months
	1	17 months
	1	14 months
	3	13 months
	4	12 months
	4	11 months
Dictyuchus	1	24 months
	1	17 months
Isoachlya	3	15 months
	4	13 months
Leptolegnia	1	30 months
	1	12 months
Pythiopsis	2	15 months
	5	13 months
	1	12 months
Saprolegnia	1	30 months
	6	24 months
	4	13 months
	4	12 months
	4	10 months
Thraustotheca	1	12 months
	4	11 months

to grow on being subcultured, seven because their revival was long delayed, and assumed or observed to be the result of the germination of oospores: other slants of the same isolates have now, however, retained viability under oil for longer periods. It is suggested that a possible reason for these early failures lies in the use of unsupplemented corn-meal agar, on which *Achlya* and *Brevilegnia* in particular showed relatively slow growth. In May, 1948, subcultures were made of the 133 slants of the remaining sixty-seven isolates on corn-meal agar (forty-four isolates were maintained as single slants, twenty-three isolates as 2–6 slants each) which had been under mineral oil for varying periods, with the results shown in table 1. All were viable.

These results show that the mineral oil method can be successfully applied to the preservation of these delicate fungi.

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CONIDIA-LIKE STRUCTURES IN PLECTANIA COCCINEA

CONST. J. ALEXOPOULOS AND ED. E. BUTLER

(WITH 1 FIGURE)

On May 3, 1947 several ascocarps of *Plectania coccinea* (Scop.) Fuckel were collected in Sanford woodlot, East Lansing, Michigan. Upon microscopic examination of ascospores scraped off the hymenial layer, it was found that a number of them were already germinating by means of one to four germ tubes each of which cut off a conidium-like cell at the tip. The germ tubes varied in length from approximately half the length of the ascospore to three times that length. A number of detached "conidia" were also found in the same mount.

An attempt was made to induce this phenomenon by germinating ascospores on agar and in water. On May 5, ascospores were caught on the surface of potato dextrose agar in a Petri dish inverted over a puffing apothecium which had been kept in a moist chamber for two days. Several ascospores germinated, but no "conidia" were formed. Spores from a puffing apothecium caught on a dry glass slide subsequently placed in a moist chamber failed to germinate.

An apothecium collected on May 13 was placed in a moist chamber. On May 15 ascospores were caught on a glass slide and mounted under a cover glass in distilled water. The slide, supported on two pieces of glass rodding, was placed in a Petri dish into which some water was poured. In 24 hours numerous ascospores situated near the edge of the cover glass had germinated by means of one to four germ tubes. The percentage of germinated ascospores diminished in proportion to their distance from the edge of the cover glass. No conidia-like bodies had been formed as yet, but typical constrictions in the germ tubes some distance from the tip suggested that they were in the process of formation. Indeed on May 17, 45 hours after ascospore ejection from the asci, numerous "conidia" were observed completely formed at the tips of the germ tubes.

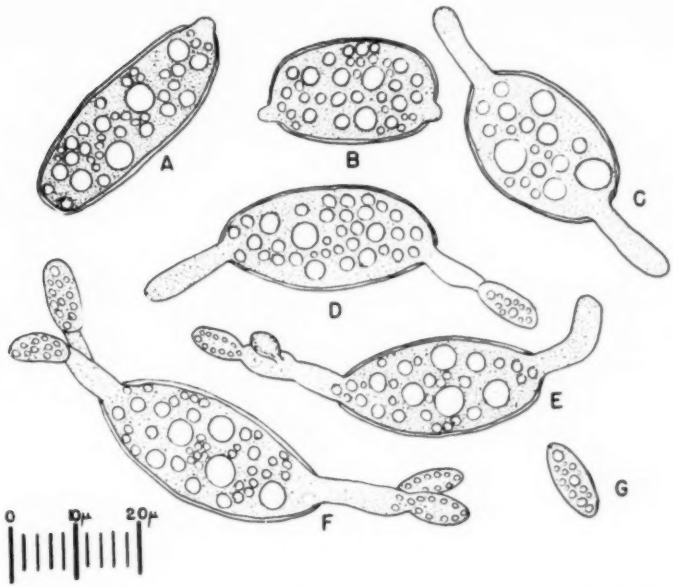

FIG. 1. *Plectania coccinea*. (A–C) Germinating ascospores. (D–F) Formation of conidia-like structures. (G) Mature "conidium."

Collections from three localities near East Lansing were made and observations were repeated in the spring of 1948 when the ascocarps of *Plectania coccinea* again made their appearance. Scrapings off the surface of the hymenium always yielded ascospores with germ tubes and "conidia" (FIG. 1). If the apothecia were permitted to remain in a moist chamber for a few days, the number of germinated ascospores and especially of detached "conidia" increased greatly. "Conidia" were invariably produced by the time the germ tube had grown to half the length of the ascospore. In some cases "conidia" were found budding directly from the ascospore.

One apothecium was left in a moist chamber for a period of two weeks. Scrapings off the hymenial layer were mounted in water at the end of that period. The hymenium was in a state of advanced disintegration, but a very large number of the conidia-like cells were evident among various contaminating organisms. All ascospores had completely disintegrated.

The conidia-like structures produced by *Plectania coccinea* resemble the ascospores of this fungus closely except for their size. The average size of the ascospores in the material at hand was found to be 14.5 × 31.5 μ while the average size of the "conidia" was 4.5 × 11 μ. The range in size of the "conidia" was 3.7–5 × 9–13 μ.

The exact nature of the conidia-like cells abundantly produced by *Plectania coccinea*, as reported above, is not clear. Germination has not been observed in any of the preparations in which these cells were found, but no extensive germination studies were undertaken.

Similar structures arising in the same way from germinating ascospores of *Peziza vesiculosa* and *Bulgaria inquinans* were observed in 1865 by the Tulasne brothers (3). Copious "conidiola" were observed on short, thick germ tubes arising from the ascospores. Brefeld (2) records production of similar structures he calls conidia, from germinating ascospores of *Coryne sarcoides*, but not from *Plectania coccinea* of which fungus he says, ". . . brachte nur unfruchtbare weisse, üppige Mycelien." He does not describe the germination of the ascospores in the latter species.

Budding of ascospores of several species of ascomycetes is a well known phenomenon. In the Taphrinales and yeasts budding is of common occurrence. Bond (1), who describes the production of sprout cells from the ascospores of *Sphaerulina mappiae*, gives a brief review of the literature on budding ascospores.

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CREBROTHECIUM ASHBYI [1]

JOHN B. ROUTIEN [2]

During the course of a review of the literature on *Spermophthora, Eremothecium, Ashbya* and *Nematospora* the writer became aware of the fact that though the first species described for the genus *Eremothecium, E. Cymbalariae* Borzi, had been validly published (**2**), another name, *E. Ashbyi,* had been proposed first in 1935 (**3**) for a second fungus but had not been published validly according to strict interpretation of Article 38 of the International Rules of Botanical Nomenclature (**5**) in that the description was published after January 1, 1935 and, unfortunately, was not accompanied by a Latin diagnosis. Though many mycologists would disregard such a point, it seems to the writer that the description of the fungus in question should be published effectively and validly without more delay.

Guilliermond (**3, 4**) considered that the fungus he was describing best fitted into the genus *Eremothecium.* There were many similarities between his fungus and *E. Cymbalariae,* it is true: The manner of growth was the same in each; asci (or sporangia ?) arose in each in a similar manner from multinucleate cells; the spores had the same shape. However, there were several ways in which the new fungus differed from *E. Cymbalariae:* in *Eremothecium* the asci were described as solitary at the apices of hyphae, lageniform or nearly so, with 30 or more spores in each, and the spores were arranged in two clusters at opposite ends of the ascus; in the new fungus, on the other hand, the asci usually were arranged in a series of several in an intercalary position and only occasionally solitary, they were ellipsoidal-truncate, there were 4–32, mostly 6–18 and rarely fewer than 4 or more than 32, spores in

[1] Contribution from the Mycology Laboratory, Chas. Pfizer and Co., Brooklyn, N. Y.

[2] The author hereby acknowledges the kind, invaluable assistance of Dr. E. A. Bessey during the preparation of this paper. His thanks also go to Misses Josephine LaBruna and Louise Sarno for translation of Borzi's original paper.

each sporogenous cell, and though the spores sometimes showed a vague clustering they were always entangled and overlapped one another.

In some ways it might be best merely to validate the combination *Eremothecium Ashbyi*, but the writer believes that the differences enumerated in the previous paragraph are so great that the two fungi should be placed in different genera.

The best classification of these two fungi seems to the author to be to regard them as non-sexual forms derived from a *Spermophthora*-like organism and to place all three in the same family. Guilliermond (**4**) reported that a culture of *S. Gossypii* after 8 years of cultivation had ceased to reproduce sexually but had a form of asexual reproduction which basically was the same as that demonstrated by the two organisms here discussed. Moreover, Ashby and Nowell (**1**) had stated earlier that in *S. Gossypii* "The spores germinate by germ-tubes which (1) develop into the primary mycelium or (2) fuse in pairs. . . ." It seems quite possible, then, that forms like *Eremothecium* could easily be derived from a form like *Spermophthora* by a disappearance of sexual reproduction.

The name *Crebrothecium* is derived from *creber* (frequent, numerous or repeated), in reference to the numerous sporogenous cells, and *theca*.

Crebrothecium gen. nov.

Hyphis dichotome ramosis; cellulis fertilis truncato-ellipsoideis, saepius catenatis sed interdum singularibus; pariete asci deliquescente; ascosporis saepius 8–16 in asco quoque, sed interdum paucis vel multis, irregulatim dispersis vel in fasciculis indefinitivis, clavato-acicularis, rectis vel saepe curvulis, continuis, una parte sporae vacua et fastigata; ex sporis germinantibus oritur mycelium primigenium.

Hyphae branched dichotomously; sporogenous cells truncate-ellipsoidal, usually in chains but occasionally solitary; wall of ascus becoming deliquescent; spores mostly 8–16 in each ascus, but occasionally few or more, irregularly disposed or sometimes in vague clusters, clavate-acicular, straight or often curved, simple, a part of the spore devoid of contents and attenuated to a pointed prolongation; spores germinating to produce primary mycelium.

The type species is *Crebrothecium Ashbyi* (Guill.) Routien.

C. Ashbyi (Guill.) comb. nov.

Mycelio postremo lutescente; hyphis 2.7–5.5 μ latis; ascis 68–87 $\times$ 14–16 μ; sporis 29–31.8 $\times$ 2–2.8 μ. Parasito in capsulis *Gossypii* in Sudan.

Mycelium becoming yellow; hyphae 2.7–5.5 μ wide; sporogenous cells 68–87 $\times$ 14–16 μ; spores 29–31.8 $\times$ 2–2.8 μ. Parasitic in capsules of *Gossypium* in Sudan.

Type: Figures 1, 6, 7, 13, 18, 19 in Guilliermond (**4**).

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THE INFLUENCE OF CONCENTRATIONS OF NUTRIENTS, THIAMIN, AND BIOTIN UPON GROWTH, AND FORMATION OF PERITHECIA AND ASCOSPORES BY CHAETOMIUM CONVOLUTUM [1]

VIRGIL GREENE LILLY AND H. L. BARNETT

(WITH 4 FIGURES)

An adequate supply of a given vitamin has been found to be essential for fruiting by some vitamin-deficient fungi. *Sordaria fimicola* (**1**, **7**) was found to require more biotin for the formation of perithecia and ascospores than for vegetative growth. The amount of biotin required for the formation of ascospores was greater than that required for the formation of perithecia. The absolute amount of biotin required for the formation of perithecia decreased as the amount of nutrients in the medium decreased. Similar results have been reported for *Ceratostomella fimbriata* (**2**) which is deficient for thiamin. The amount of thiamin required by this fungus for the production of perithecia decreased as the amount of nutrients in the medium decreased. The numbers of perithecia produced by these two fungi depended upon the amount of nutrients present as well as upon the concentrations of the vitamins employed. Hawker (**3**) found that the concentration of glucose optimal for the fruiting of sixteen fungi increased as the concentration of growth substances increased.

It was considered desirable to extend the study of the interrelations among nutrient supply, vitamin concentrations and sexual reproduction to another fungus. *Chaetomium convolutum* Chivers was found to be deficient for both thiamin and biotin. This paper reports the interrelations between nutrient supply and concentrations of thiamin and biotin as they affect the growth and sexual reproduction of this fungus.

[1] Published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 397.

MATERIALS AND METHODS

The isolate of *C. convolutum* used was obtained from a contaminated plate. Preliminary study showed that this isolate fruited readily under certain laboratory conditions, and was deficient for thiamin and biotin.

The basal liquid medium used had the following composition:

Glucose	25 gm.
KH_2PO_4	1 gm.
$MgSO_4.7H_2O$	0.5 gm.
Fumaric acid	1.32 gm.
Casein hydrolysate equivalent to	2 gm. casein
Na_2CO_3	1.1 gm.
Fe+++	0.2 mg.
Zn++	0.2 mg.
Mn++	0.1 mg.
Double-distilled water to make	1000 ml.

Leonian and Lilly (**6**) described a method of preparing this medium in essentially vitamin-free condition. The pH of the medium was adjusted to 6.5 before autoclaving by adding sodium hydroxide or hydrochloric acid. The pH of the medium was approximately 6.0 after autoclaving. Thiamin and biotin were added to the medium before autoclaving in the amounts specified in connection with the experiments. The concentrations of the vitamins are given in micrograms (μg.) per flask.

The culture vessels used were 250 ml. pyrex Erlenmeyer flasks which had been cleansed in dichromate-sulfuric acid solution. Twenty-five ml. of medium were used per flask. Inoculation was made by adding a loopful of spore suspension to each flask. The temperature of incubation was $25 \pm 1°$ C.; the time of incubation varied and is reported in connection with the experiments.

The number of flasks prepared for each concentration studied varied from four to ten, depending upon the experiment. The amount of growth was followed by harvesting duplicate flasks and weighing the mycelium after it had been dried to constant weight. The weights are reported in milligrams, each datum being the average of two single determinations. The pH of the culture medium was determined at time of harvest by means of glass electrode assembly. Perithecia were considered present when they could be

seen as small black dots with the unaided eye. The numbers of perithecia were estimated by rough counts and the degree of maturity was determined by microscopic examination. The presence of ascospores was detected by crushing perithecia under a cover slip on a slide.

EXPERIMENTAL RESULTS

Effect of biotin and thiamin upon growth and formation of perithecia: Two extensive experiments were made to determine the effect of varying concentrations of thiamin and biotin upon growth, the pH of the culture medium, and the formation of perithecia. Since exogenous thiamin and biotin are required by this fungus, it was necessary to keep the concentration of one vitamin constant while the concentration of the other varied. A portion of the data will be presented in the form of graphs.

In the first experiment, the concentration of thiamin was kept constant (2.5 μg. per flask) while the concentration of biotin varied

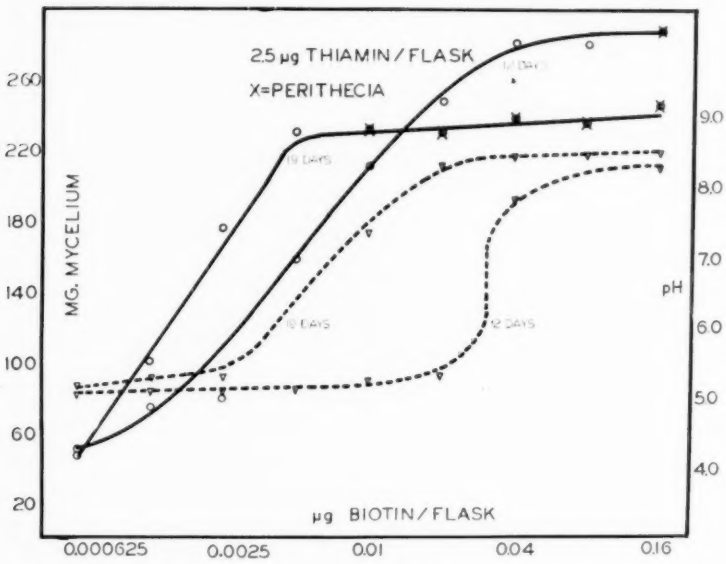

FIG. 1. Effect of varying concentrations of biotin on growth, pH of the culture medium and formation of perithecia at 12 and 19 days of incubation. Undiluted medium. ————, growth; - - - - - - - -, pH; X, perithecia.

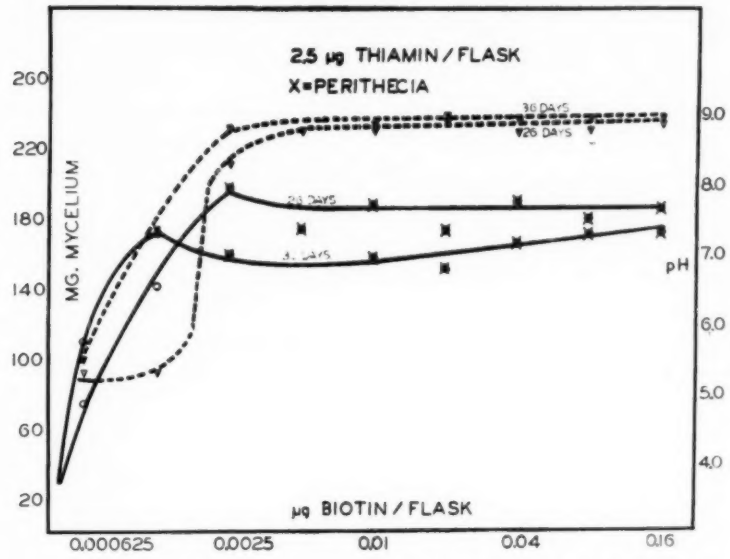

FIG. 2. Effect of varying concentrations of biotin on growth, pH of the culture medium and formation of perithecia at 26 and 36 days of incubation. Undiluted medium. ————, growth; - - - - - - - -, pH; X, perithecia.

from 0.0 to 0.16 µg. per flask. Figures 1 and 2 show that growth was proportional to the amount of biotin in the medium when the concentration of biotin was less than 0.04 µg. The time of incubation required to produce the maximum weight of mycelium with the lower concentrations of biotin increased as the concentration of biotin decreased. Autolysis followed soon after maximum weight was attained. The pH of the culture medium remained near 5 during early growth; as the weight of mycelium approached a maximum, the pH increased to 8–9. Presumably this increase in pH is connected with the process of autolysis.

The concentration of biotin influenced the time of incubation required for perithecia to form, and to some extent the numbers produced. While a concentration of biotin greater than 0.04 µg. had but little effect upon growth, these higher concentrations were more effective in the rapid production of perithecia. Thus perithecia were produced by the twelfth day when the concentration of biotin was 0.16 µg., whereas fifteen days were required when the concen-

tration was 0.08 and 0.04 μg. The time of appearance of perithecia in the same series of flasks varied somewhat; the time of perithecial formation recorded in figures 1 and 2 is that found when half or more of the flasks in a given experiment exhibited perithecia visible to the unaided eye. This variation in time of perithecial formation was seldom greater than one day when the concentrations of biotin were greater than 0.04 μg. As the concentration of biotin was decreased further, this variation increased to as much as a week (lowest concentrations). The pH of the culture medium was always greater than 7 when perithecia formed. However, the flasks receiving 0.00625 μg. of biotin did not produce perithecia after 49 days of incubation and the pH was 7.57 at this time.

The effects of varying concentrations of thiamin in the presence of 0.16 μg. biotin upon growth, the pH of the culture medium, and on perithecial formation are presented in figures 3 and 4. The amount of growth was proportional to the concentration of thiamin

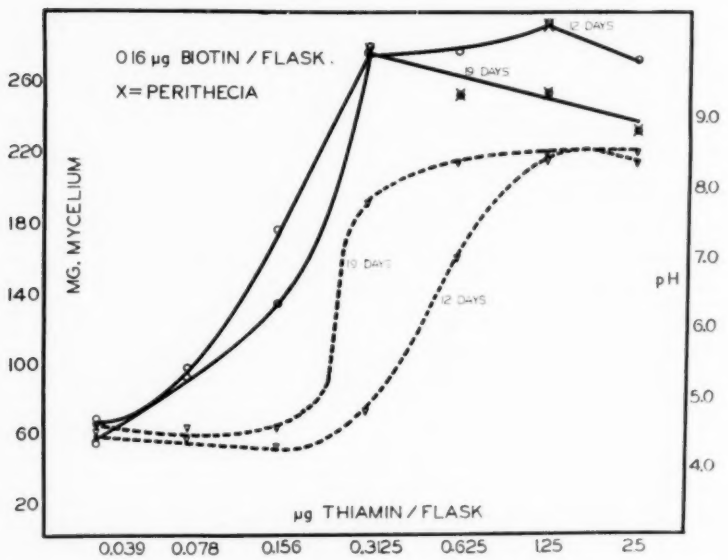

FIG. 3. Effect of varying concentrations of thiamin on growth, pH of the culture medium and formation of perithecia at 12 and 19 days of incubation. Undiluted medium. ————, growth; - - - - - - - -, pH; X, perithecia.

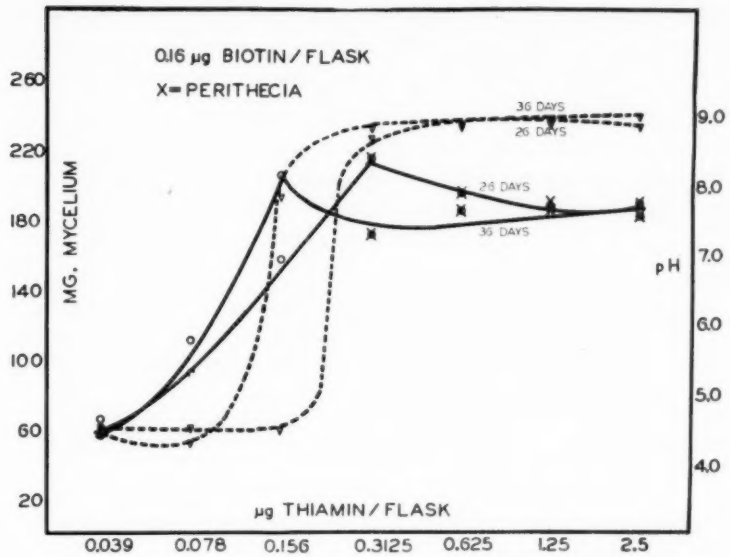

FIG. 4. Effect of varying concentrations of thiamin on growth, pH of the culture medium and formation of perithecia at 26 and 36 days of incubation. Undiluted medium. ————, growth; - - - - - - - -, pH; X, perithecia.

in the medium in the range below 0.3125 µg. Concentration of thiamin greater than this had but little effect on the amount of mycelium produced. However, the formation of perithecia was induced sooner in the higher concentrations of thiamin. While the changes in the pH of the culture medium resembled those in the biotin-series, it may be noted that in the lower thiamin concentrations the pH fell almost one unit below that of the biotin series. In general, the change in pH to the alkaline range was accompanied by a rapid increase in the rate of growth.

Dilution of the medium and the absolute amounts of the vitamins required for the formation of perithecia and ascospores: The experiments described above employed full strength media. The results showed that, for a given period of incubation, there were critical concentrations of thiamin and biotin below which perithecia did not form. The following experiments were made to determine if the amount of nutrients in the medium affected these criti-

cal concentrations. Full strength medium and one-fourth and one-sixteenth dilutions were used. In order to lessen the number of flasks required, only the lower concentrations of thiamin and biotin were used. Harvests were made at the time when perithecia were first observed. Some of the flasks were reserved so that microscopic examination could be made of the perithecia and ascospores. The results of this examination are given in tables 1 and 2.

TABLE 1

THE EFFECTS OF VARYING CONCENTRATIONS OF BIOTIN AND NUTRIENTS UPON GROWTH, pH OF THE CULTURE MEDIUM, AND THE NUMBERS AND DEGREES OF MATURITY OF PERITHECIA FORMED.

2.5 μg. thiamin hydrochloride per flask

μg. biotin/flask	mg. mycelium	pH at harvest	Days incubation to form perithecia	Total number of perithecia formed and per cent mature
Undiluted Medium				
0.0	1	6.0	None at 60 days	None
0.0025	161	7.67	46	12,000, none mature, no ascospores
0.005	204	8.66	28	10,000, few mature, only an occasional ascospore
0.01	194	8.78	28	15,000, 25% mature
$\frac{1}{4}$ Dilution				
0.0	1	6.45	None at 60 days	None
0.000625	55	7.56	30	2000, 65% mature
0.0025	68	7.80	20	4000, 80% mature
0.005	67	7.61	15	5000, 85% mature
$\frac{1}{16}$ Dilution				
0.0	1	6.45	None at 60 days	None
0.0003125	15	7.23	17	500–700, less than 25% mature
0.000625	16	7.90	15	500–700, 50% mature
0.00125	21	7.94	12	500–700, 65% mature

It may be seen from tables 1 and 2 that the formation of perithecia by *C. convolutum* is dependent upon at least three factors: the concentration of thiamin, biotin, and of nutrients. For each concentration of nutrients there is a fixed critical concentration of thiamin and biotin below which perithecia do not form. A given

TABLE 2

THE EFFECTS OF VARYING CONCENTRATIONS OF THIAMIN HYDROCHLORIDE AND NUTRIENTS UPON GROWTH, pH OF THE CULTURE MEDIUM, AND THE NUMBERS AND DEGREE OF MATURITY OF PERITHECIA FORMED.

0.16 μg. biotin per flask

μg. thiamin/flask	mg. mycelium	pH at harvest	Days incubation to form perithecia	Total number of perithecia formed and per cent mature
Undiluted Medium				
0.0	1	5.05	None at 60 days	None
0.078	171	4.92	None at 60 days	None
0.156	149	8.41	54	10,000, none mature, no ascospores
0.3125	220	8.00	24	10,000, few mature, few ascospores
0.625	220	7.96	17	13,000, 25% mature
¼ Dilution				
0.0	3	5.55	None at 60 days	None
0.039	44	7.96	60	1500–3000, 80% mature
0.078	58	8.50	28	2100–3000, 80–90% mature
0.156	75	8.12	12	3000, 90% mature
1/16 Dilution				
0.0	1	5.10	None at 60 days	None
0.0195	17	7.81	15	400–500, 50% mature
0.039	22	7.94	12	400–500, 50% mature

concentration of the vitamins may be above or below the critical concentrations, depending upon the concentration of nutrients in the medium. In general, the time of incubation required to produce perithecia in the presence of fixed amounts of the vitamins decreases as the medium becomes more dilute. Thus, the days required for perithecia to form when the biotin concentration was 0.025 μg. decreased from 46 to 20 and 12 days, respectively, when the medium was diluted to one-fourth and one-sixteenth full strength. However, perithecia did not form under any condition tried in less than ten days. The formation of perithecia appears to be connected with exhaustion of available nutrients.

A microscopic examination of the perithecia formed under the conditions of the previous experiment was made on the sixtieth day of incubation. At that time, the numbers of perithecia were

estimated by rough counts, and the maturity of the perithecia determined by examination. A perithecium was judged mature if it contained ascospores (the asci had degenerated in most instances by the time the examination was made). The relative number of mature and immature perithecia were estimated by rough counts under the microscope. The relevant data are given in tables 1 and 2.

DISCUSSION

In the presence of a given amount of nutrients (thiamin and biotin present) vegetative growth takes place first. The amount of vegetative growth is controlled by the amount of nutrients present and by the concentrations of thiamin and biotin. Only as vegetative growth attains a maximum do perithecia form. When the concentration of nutrients is the factor limiting growth, perithecia form only after the attainment of maximum growth. For any concentration of nutrients certain concentrations of thiamin and biotin are required before mature perithecia develop. Lower concentrations of these two vitamins allow perithecia to form, but these may never mature and form ascospores. At still lower concentrations of these vitamins perithecia do not form at all.

As the amount of nutrients in the medium is reduced lower concentrations of thiamin and biotin are required for sexual reproduction. Under these conditions the amount of vegetative growth is reduced and it may be deduced that each unit weight of mycelium must contain certain concentrations of these vitamins before sexual reproduction is possible. Therefore, the *relative* amounts of nutrients and thiamin and biotin determine whether perithecia and ascospores form and also control the time of incubation required for their production.

The general conclusions reached in our studies on the factors controlling sexual reproduction of *Sordaria fimicola* and *Ceratostomella fimbriata* were confirmed in this study of *Chaetomium convolutum*. More work will be required to determine the general validity of these conclusions with regard to the sexual reproduction of other vitamin-deficient fungi. It is to be expected that other nutritional factors may prove to be of equal importance as other fungi are studied.

Hawker (**4**) has found nutritional factors in addition to the vitamins to be of great importance for the fruiting of *Melanospora destruens*. Not only the concentration of the sugar, but the kind used in the medium, was of great importance for the production of perithecia by this species. Thus, 50 grams of sucrose per liter allowed the formation of numerous perithecia; none were formed under comparable conditions when the same weight of glucose was used. A few perithecia formed when the amount of glucose was reduced to 5 grams per liter. On the other hand, glucose supported greater vegetative growth of *M. destruens* than sucrose.

Klebs (**5**) fully recognized the role of specific constituents as well as concentration of the medium in reproduction by the fungi. His generalization that reproduction takes place under more restricted environmental conditions than growth is confirmed in this paper.

SUMMARY

Chaetomium convolutum is deficient for both thiamin and biotin. In the presence of an optimum amount of one of these vitamins growth was proportional to the amount of the other vitamin in the medium. The formation of perithecia and ascospores followed the attainment of maximum vegetative growth provided that the concentrations of these vitamins exceeded certain values. The absolute amounts of thiamin and biotin required for the formation of perithecia and ascospores were a function of the concentration of the nutrients in the medium. The numbers of perithecia formed for a given concentration of nutrients increased somewhat as the concentrations of the vitamins were increased, the numbers of mature perithecia formed were more nearly proportional to the concentrations of the vitamins. This was also true of the numbers of ascospores formed. The amounts of thiamin and biotin required for vegetative growth, perithecial formation and the production of ascospores in the presence of a given amount of nutrients increased in the above order.

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DOWNY MILDEW OF URTICA IN THE UNITED STATES [1]

CHARLES GARDNER SHAW [2]

(WITH 1 FIGURE)

Berkeley (**2**) in 1846 was the first to mention the occurrence of a downy mildew on a species of *Urtica*. He compared *Botrytis Urticae* Libert (evidently a manuscript name) and *B. infestans* Mont. [= *Phytopthora infestans* (Mont.) de Bary], and concluded that the two were distinct. He failed, however, to characterize the former species adequately. Five years later, Berkeley and Broome (**4**) gave a more nearly complete description of *B. Urticae*. They described the conidia as "large, ovate, apex papillaeform," and gave the host as "common nettle" (undoubtedly referring to *U. dioica* L.).

Caspary (**6**) in 1855 transferred this species to the genus *Peronospora*, but failed to give a description or a reference to another description. Berkeley (**3**) in 1860 accepted this transfer and used the binomial *P. Urticae* Casp. for the fungus.

Next, de Bary (**1**), in his monograph of the family "Peronosporei," described a downy mildew found on *Urtica urens* L. which had apically rounded, basally pedicellate conidia. Oospores typical of his section Effusae were also observed. Although he applied the name "*Peronospora Urticae* (Lib.)" to his material, de Bary stated that Berkeley and Broome's description did not agree with his.

[1] Based on a portion of a thesis submitted to the graduate school of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Assistant Professor and Assistant Pathologist, Division of Plant Pathology, Washington State College.

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Subsequent workers, including Fischer (**8**), Berlese (**5**), Gäumann (**9**), and many others, consistently listed both species of *Urtica* as hosts of *P. Urticae*.

Salmon and Ware (**12**) noted zoospore germination of conidia produced on *U. dioica* and therefore made the combination *Pseudoperonospora Urticae* (Lib.) Salm. and Ware. After additional study, these workers (**13**) pointed out that two distinct downy mildews occur on species of *Urtica*. One, to which they applied the binomial *Pseudoper. Urticae* (Lib.) Salm. and Ware, has apically papillate and poroid, colored conidia which germinate by the formation of zoospores. It usually occurs on *U. dioica*, but has also been found on *U. urens*. The other, to which they apply the binomial *Per. deBaryi* Salm. and Ware, has apically rounded, basally pedicellate, colored conidia which germinate by means of a germ tube. This one occurs on *U. urens*.

In the United States, the first report of a downy mildew on *Urtica* was made in 1880 by Harkness and Moore (**10**), who applied the binomial *Per. Urticae* to it. Next, Trelease (**15**) reported the occurrence of "*Peronospora urticae* (Lib.)" on *Urtica gracilis* Ait. from two localities in Wisconsin. These two published records appear to be the only reports of a downy mildew on *Urtica* in the United States. The listings in Seymour's (**14**) and Wilson's (**16**) host indices are apparently based on these reports. In the light of the findings of Salmon and Ware, it seemed advisable to recheck all available material to determine the identity of the fungus present in the United States.

By personal correspondence, Lee Bonar has informed the writer that, except for a few type specimens, the collections made by Harkness and Moore were destroyed in the San Francisco earthquake and fire. It would appear that no portion of their collection is still extant.

Carroll W. Dodge of the Missouri Botanical Garden kindly loaned the author the following material: "*Peronospora Urticae* (Lib.) on *Urtica;* Kirkland (now Devil's Lake, Sauk Co.), Wis.; June, 1884. Pammel." The second collection, originally reported as collected at La Crosse, Wisconsin (**15**), is no longer represented

in the Trelease herbarium. The Kirkland specimen consists of two leaves abundantly covered on the lower surface with a cinereous mat of conidiophores.

An attempt to locate additional specimens proved unsuccessful. D. P. Rogers loaned the author two packets of the following collection: "*Peronospora Urticae* (Lib.) D By. on remains of *Aecidium Urticae* on *Urtica gracilis*. Glenwood, Minnesota; B. C. Taylor; July, 1891." A third portion of this collection reposes in the University of Wisconsin Cryptogamic Herbarium. Careful examination of all three specimens failed to disclose the presence of the downy mildew. Notes on the two packets from the New York Botanical Garden indicate that G. W. Wilson was also unable to find a downy mildew present.

Lee Bonar loaned the following specimen: "*Peronospora Urticae* D By. on *Urtica Lyallii;* Marysville, Wash.; July, 1927; J. M. Grant; U. Calif., Berkeley, Herb. No. 438288." Again a careful examination failed to reveal the presence of a downy mildew.

G. R. Hoerner, who has reported successful experimental infection of *Urtica* spp. with *Pseudoperonospora Humuli* (Miyabe & Takah.) Wilson from *Humulus Lupulus* L. (**11**), has informed the writer, in personal correspondence, that he has never found a downy mildew on any species of *Urtica*, and has no specimens of naturally infected species.

Since the collection by Pammel in the Trelease herbarium seems to be the only United States specimen extant, a description of that specimen is given below:

PERONOSPORA DEBARYI Salm. & Ware. Figure 1

Foliicolous; infected area angular, becoming necrotic and light brown; thin, cinereous mat below; conidiophores hypophyllous, aseptate, hyaline or slightly tinted, emerging singly or in groups of 2–5 from a stoma, 4–6 times subdichotomously branched, 200–460 × 5–8 μ; crown about ½ the total height, main branches somewhat curved, arising at acute angles; ultimate branches acute to subacute, rather short, 4–9 μ, a few up to 15 μ long, the axial longer, straight or slightly incurved, the abaxial straight or occasionally slightly recurved; conidia light brown, broadly ellipsoid,

obtusely rounded apically, basally pedicellate, 19–27 × 13–19 μ (mean 22.42 × 16.11 μ); oospores not observed.

On Urticaceae: Conidia on *Urtica gracilis* Ait.

Collected in Wisconsin in June.

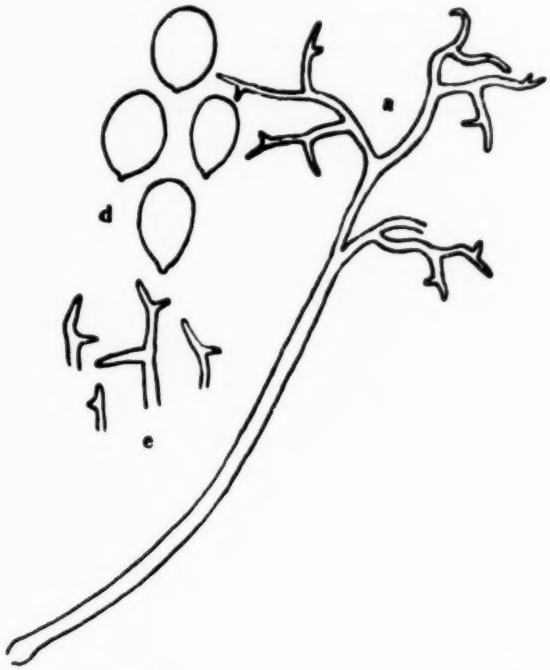

FIG. 1. *Peronospora deBaryi*—drawn with the aid of a camera lucida. *a*, conidiophore—× 300; *d*, conidia—× 375; *e*, ultimate branches—× 375.

The above description agrees in all essentials with Salmon and Ware's description of *P. deBaryi*. The conidia of the American specimen are somewhat smaller than are those described by Salmon and Ware. Otherwise the American material is very similar. Because we have only one specimen, which lacks oospores, and because of the variability known to occur in the conidia of many downy mildews (**7**), the American material is at present considered to belong in *Peronospora deBaryi* Salm. and Ware.

The writer wishes to express his thanks to those who have loaned him specimens.

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THE HELMINTHOSPORIUM ON BUCHLOE DACTYLOIDES

C. L. Lefebvre and A. G. Johnson [1]

(with 3 figures)

A characteristic species of *Helminthosporium* was collected on buffalo grass [*Buchloe dactyloides* (Nutt.) Engelm.] at Stockton, Kansas, on July 24, 1895, by Elam Bartholomew, his No. *1799*. He labelled the specimen (in Herb.) "*Helminthosporium inconspicuum* C. & E. var. *Buchloes* E. & B. n. var." Bartholomew supplied specimens of this same collection to J. B. Ellis who issued them in 1896 as extra numbers with Ellis and Everhart, North American Fungi, Century 34, as No. *45* (b), and with their Fungi Columbiani, Century 9, as No. *298* (b). The former is labelled "*Helminthosporium inconspicuum* C. E. var. *Buchloes*," and the latter "*Helminthosporium inconspicuum* C. & E. var. *Buchloes*." Subsequently, this fungus has been referred to as "*H. inconspicuum* C. & E. var. *Buchloes* E. and Ev." (**1**, **2**). "*H. inconspicuum buchloes* Ell. and Ev." (**3**), or simply as "*H. inconspicuum* var. *Buchloes*" (**4**).

In addition to examining the exsiccati specimens referred to above, six more recent collections of the same fungus on the same host, referred to later, have been examined. In all of these, the same characteristic fungus was found. Furthermore, the fungus was found to differ distinctly from that originally described on corn as *Helminthosporium inconspicuum* Cke. and Ell., now generally known as *H. turcicum* Pass. (**5**). The striking differences in the way the conidiophores are produced in the two fungi and in the shape, size, and hilum characters of their conidia are indicated in table 1, and differences in spore characters are shown in figures *2* and *3*.

[1] The writers are grateful to Edith K. Cash for assistance in preparing the Latin description of the fungus on buffalo grass; to J. A. Stevenson for counsel in connection with these studies; and to W. Lawrence White for assistance in determining the date of issuance of the exsiccati specimens.

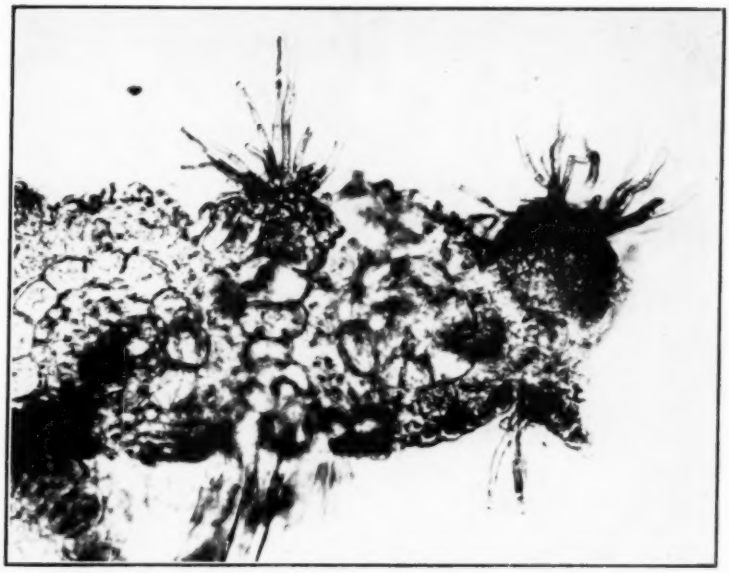

FIG. 1. Cross section of leaf of *Buchloe dactyloides* with groups of conidiophores and stromata of *Helminthosporium buchloes* between fibrovascular bundles. × 250.

TABLE 1

COMPARISON OF DIAGNOSTIC CHARACTERS OF *Helminthosporium turcicum* AND THOSE OF THE SPECIES OF *Helminthosporium* ON BUFFALO GRASS

Characters Compared	*Helminthosporium turcicum*	The *Helminthosporium* on Buffalo Grass
Conidiophores	Mostly in small groups, not on stromata.	Chiefly in large groups, on stromata.
Conidia		
Shape	Tapering about equally toward tip and base, base rather acute.	Tapering toward apex, base rounded.
Size	15–25 × 45–132 μ	8–11 × 27–86 μ
Hilum	Conspicuous, protruding.	Inconspicuous, not protruding.

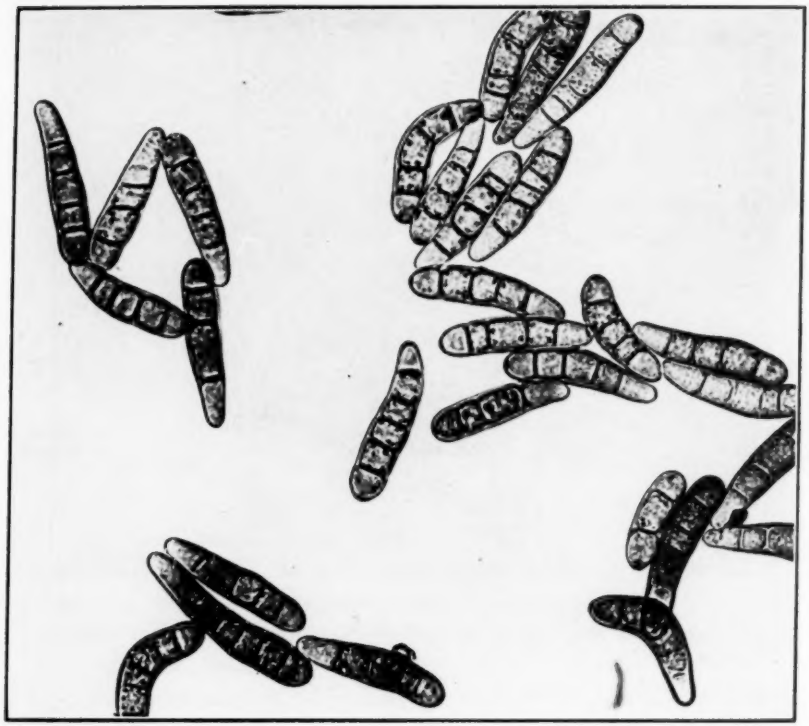

FIG. 2. Conidia of *Helminthosporium buchloes* developed in a moist chamber April 13, 1948, on an infected leaf of *Buchloe dactyloides* collected at Hays, Kansas, September 28, 1947. × 500.

Because of these differences, it seems clear that the fungus on buffalo grass does not belong as a variety under *H. inconspicuum* Cke. and Ell., but rather deserves specific rank. A search of the literature indicates that the variety *Helminthosporium inconspicuum* var. *buchloes* Ell. and Ev. has never been described. This name, therefore, is a *nomen nudum*. Likewise the name "*H. inconspicuum* var. *buchloes* E. & B." (in Herb.) is a *nomen nudum*. Because of these considerations, the fungus on buffalo grass is here described as:

Helminthosporium buchloes sp. nov.

Maculis primum flavis vel rubro-flavis, demum stramineis ex parte obscuris; conidiophoris singulatim vel 2–20 caespitosis, olivaceo-brunneis,

apices versus pallidioribus, 2–9-septatis, 5–8 μ diam., 60–120 μ longis; conidiis subhyalinis usque pallide-fuliginosis in aetate obscurescentibus, 2–9-septatis, rectis curvatisve, interdum subsinuosis, ad apicem rotundatum attenuatis, basi rotundatis, hilo inconspicuo praeditis, tubulis duobus polaribus germinantibus, 8–11 μ diam., 27–86 μ longis.

In foliis *Buchloes dactyloidis* in U. S. A.

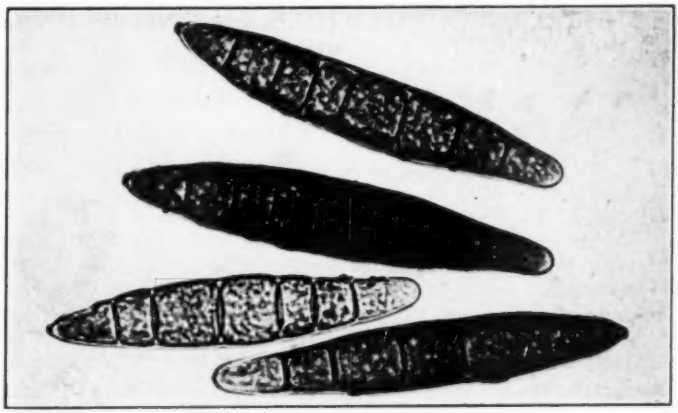

FIG. 3. Conidia of *Helminthosporium turcicum* Pass. (*H. inconspicuum* Cke. and Ell.) developed in a moist chamber June 2, 1948, on an infected leaf of corn (*Zea mays* L.) collected by Merle T. Jenkins near Cincinnati, Ohio, September 7, 1947. × 500.

Spots on leaves at first yellow to reddish-yellow, later bleaching to straw-color with dark blotches on both sides of leaf as stromata and conidiophores are produced. Conidiophores arising singly or in groups of two to twenty or more, the larger groups being produced from brown stromata between the fibrovascular bundles (FIG. 1); conidiophores olivaceous-brown, lighter toward apices, 2–9-septate, 5–8 μ in diameter at the basal septum, and 60–120 μ long; conidia subhyaline to light fuliginous becoming darker when older, 2–9-septate, straight or curved, occasionally tending to be somewhat sinuous, tapering toward the rounded apex, basal cell rounded, with hilum inconspicuous, germinating by two polar germ tubes, 8–11 × 27–86 μ (means 9.7 × 51.8 μ) (FIG. 2).

On leaves of *Buchloe dactyloides* (Nutt.) Engelm.

Collected by: Elam Bartholomew, Stockton, Kans., July 24, 1895; C. L. Lefebvre, Manhattan, Kansas, November 7, 1938; Mildred Pladeck, Maverick Co., Texas, August 11, 1939; R.

Sprague, Lincoln, Nebraska, September 12, 1940; C. L. Lefebvre, Woodward, Oklahoma, June 13, 1942; C. L. Lefebvre, Hays, Kansas, June 18, 1942 (**type**); J. L. Allison, Madison, Wisconsin, July, 1943; C. L. Lefebvre, Hays, Kansas, September 28, 1947.

Specimens of the type collection have been deposited in the Mycological Collections of the Bureau of Plant Industry, Beltsville, Maryland, and the Farlow Herbarium, Cambridge, Massachusetts.

PLANT INDUSTRY STATION,
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NOTES AND BRIEF ARTICLES

Mycological Society of America
Report of the 1947 Foray

The second in the new series of fungus forays of the Mycological Society of America was held at Highlands, North Carolina, September 2–7, 1947 under the leadership of Dr. Julian H. Miller, vice-president. Through the courtesy of Professor Thelma Howell, resident director, and the other officers, the Highlands Biological Laboratory was made available to those attending the foray as a base for collecting operations. Working space, driers, and other facilities were freely placed at the disposal of the mycologists.

On the afternoon of Sept. 2, Dr. L. S. Olive led the group along the trail to Sunset Rock on the Ravenel Estate with collecting along the way. September 3 was devoted to an all day trip to Rabun Bald, an interesting botanical area in Rabun County, Georgia. The mycological possibilities of a bog in which ecological studies were in progress were investigated enroute. An expedition to the Upper White Water Falls in Transylvania County occupied the morning of the 4th, and the two succeeding mornings were given over to collecting along Rhododendron and Kelsey Trails in the immediate vicinity of the Biological Laboratory. The afternoons were available for the study and care of the collections. As in the previous foray in West Virginia dry weather had prevented abundant production of fleshy forms, but sufficient fungi in other groups were found to make the occasion a success.

Dr. W. A. Campbell exhibited a most interesting series of kodachrome pictures of the larger fungi at the Laboratory on Wednesday evening, Sept. 3, and an equally instructive series of kodachromes of the local flora was presented on Friday evening. On Thursday afternoon the visiting ladies were taken by Professor Howell and her associates on a tour to High Hampton Inn and a number of estates in and about Highlands to view the floral effects.

The group as a whole attended an out-of-doors gathering at the Ravenel Estate during the early evening of September 5.

An informal dinner meeting was held at the Hotel Edwards on Thursday evening with approximately forty in attendance, including as guests Professor Howell and several of the Trustees of the Museum and Laboratory. The opportunity was improved upon to express the thanks of the Society and the members present for the courtesies and helpful cooperation extended by Professor Howell and her associates.

The following attended the foray: C. H. Arndt, Ernst Bessey, Lawrence G. Burk, Dr. and Mrs. H. H. Caldwell, W. A. Campbell, Edith K. Cash, A. Hugh Dempsey, W. W. Diehl, Chas. H. Driver, Marlin A. Espenshade, Francine E. Fisher, Dr. and Mrs. L. W. R. Jackson, Richard P. Korf, Morten and Bodil Lange, Daniel M. Lynch, Julian H. Miller, Dr. and Mrs. Lindsay S. Olive, Clark T. Rogerson, Dr. and Mrs. J. B. Routien, C. L. Shear, Dr. and Mrs Walter H. Snell, Wm. C. Snyder, Mr. and Mrs. John A. Stevenson, George E. Thompson, Mr. and Mrs. M. B. Walters, and Wm. H. Worrell, Jr. It is regretted that circumstances did not permit the taking of a group picture, always an interesting feature of previous forays.

A list of species collected in so far as reported follows, symbols as listed below being used to indicate the person who made the collections, or the institution in which they are deposited:

(B) = Edith K. Cash, W. W. Diehl, C. L. Shear, John A. Stevenson, Plant Industry Station, Beltsville, Md.
(C) = R. P. Korf, C. T. Rogerson, Cornell University.
(G) = J. H. Miller and associates, University of Georgia.
(O) = L. S. Olive, Louisiana State University.
(S) = Walter H. Snell, Brown University.

MYXOMYCETES: *Ceratiomyxa fruticulosa* (Muell.) Macbr. (O); *Fuligo septica* (L.) Weber (O); *Stemonitis* spp. (O).

PHYCOMYCETES: *Synchytrium accidioides* (Pk.) Lagh. on *Amphicarpa monoica* (G).

ASCOMYCETES (except *Discomycetes*): *Anthostomella* sp. on *Solidago caesia* (G); *Balansia hypoxylon* (Pk.) Atk. on *Danthonia compressa, D. spicata* (B, G); *Bombardia fasciculata* Fr. on *Castanea dentata* (G); *Botryosphaeria ribis* (Tode ex Fr.) Gross.

& Dugg. on *Robinia pseudoacacia* (G); *Caliciopsis pinea* Pk. on *Pinus strobus* (B, G); *Ceratosphaeria* sp. (B); *Clypeolella leemingii* (Ell. & Ev.) Theiss. on *Galax aphylla* (B, G); *Cordyceps militaris* (L. ex Fr.) Lk. on insect larva (C); *C. ophioglossoides* (L. ex Fr.) Lk. on *Elaphomyces* sp. (C, G); *Creopus gelatinosus* (Tode ex Fr.) Lk. on *Rhododendron maximum* (G); *Cryptospora cinctula* (Cke. & Pk.) Sacc. on *Castanea dentata* (G); *Cucurbidothis pithyophila* (Fr.) Petr. on *Pinus strobus* (G); *Daldinia concentrica* Ces. & De N. (B); *Diatrype stigma* Hoffm. ex Fr. on *Betula* (B), on *Quercus alba* (G); *Diatrype asterostoma* Berk. & Curt. on *Nyssa sylvatica* (G); *Diatrypella favacea* (Fr.) Nits. (G); *Diaporthe leiphaemia* (Fr.) Sacc. var. *raveneliana* (Thuem. & Rehm) Wehm. on *Quercus alba* (G); *Diaporthe oncostoma* (Duby) Fckl. on *Robinia pseudoacacia* (G); *Elsinoë* sp. on *Cornus florida* (B); *Endothia parasitica* (Murr.) P. J. & H. W. Anderson on *Castanea dentata* (B, G, O); *Erysiphe polygoni* DC. on *Amphicarpa monoica, Baptisia tinctoria* (G); *Eutypella microcarpa* Ell. & Ev. on *Robinia pseudoacacia* (G); *Gibberella zeae* (Schw.) Petch on *Zea mays* (G); *Gnomonia* sp. on *Leucothoë catesbaei* (G); *Gnomonia setacea* (Pers. ex Fr.) Ces. & De N. on *Quercus maxima, Q. montana* (G); *Guignardia galactina* (Dearn. & House) Sacc. on *Galax aphylla* (G); *Hypocrea* sp. on *Acer* (B); *H. patella* Cke. & Pk. (B); *Hypoderma commune* (Fr.) Duby on Umbelliferae (B); *Hypodermopsis smilacis* (Schw.) Cash on *Smilax* sp. (B); *Hypomyces chrysospermus* Tul. on agaric (C); *H. hyalinus* (Schw.) Tul. on agaric (B, C); *Hypoxylon cohaerens* Pers. ex Fr. (B); *H. fuscum* Pers. ex Fr. on *Alnus rugosa* (G); *H. microplacum* (Berk. & Curt.) Miller on *Sassafras variifolium* (B, G); *H. multiforme* Fr. on *Betula lutea* (B); *H. punctulatum* (Berk. & Rav.) Cke. on *Quercus alba* (G); *H. rubiginosum* Pers. ex Fr. on *Betula lutea* (G), *Castanea dentata* (B); *H. serpens* Pers. ex Fr. on *Castanea dentata* (B), *Quercus alba* (G); *Leptosphaeria ogilviensis* (Berk. & Br.) Ces. & De N. (B); *L. vagabunda* Sacc. on *Azalea lutea* (G); *Lophodermium rhododendri* (Schw.) Pk. on *Rhododendron* sp. (B); *Melanomma pulvis-pyrius* (Pers. ex Fr.) Fckl. on *Castanea dentata* (G); *Metasphaeria* sp. on *Pinus strobus* (G); *Microsphaera alni* (DC.) Wint. on *Azalea*

viscosa (G); *M. alni vaccinii* (Schw.) Salmon on *Lyonia ligustrina* (B); *Mycosphaerella* sp. on *Galax aphylla* (G); *M. leucothoës* Miles on *Leucothoë catesbaei* (B, G); *Myiocopron smilacis* (De N.) Sacc. on *Smilax* sp. (B), on *S. glauca* (G); *Myriangium duriaei* Berk. & Mont. on scale insects on *Nyssa sylvatica* (G); *Nectria cinnabarina* Tode ex Fr. on *Robinia pseudoacacia* (G); *N. coccinea* Pers. ex Fr. on *Aronia melanocarpa, Castanea dentata* (G); *N. ochroleuca* (Schw.) Berk. on *Amelanchier laevis* (B, C, G); *Nectriella rousseliana* (Mont.) Sacc. (G); *Ophiodothella vaccinii* Boyd on *Polycodium candicans* (G); *Ottwia hypoxylon* (Ell. & Ev.) Shear (B); *Peckiella viridis* (Alb. & Schw.) Sacc. (C); *Phyllachora lespedezae* (Schw.) Sacc. on *Lespedeza frutescens* (G); *Phyllachora punctum* (Schw.) Orton on *Paspalum dichotomum* (B); *Pleurage albicans* (Alb. & Schw.) Griff. (C); *Rosellinia clavariae* (Tul.) Wint. on *Clavaria formosa* (G); *Sordaria appendiculata* Auersw. (G); *Stigmatea rubicola* (Ell. & Ev.) Theiss. on *Rubus flagellaris* (B, G), *R. hispidus* (G); *Uncinula salicis* DC. ex Wint. on *Salix sericea* (G); *Ustulina vulgaris* Tul. on dead wood (B); *Valsa ceratophora* Tul. on *Salix humilis* (G); *V. pini* Alb. & Schw. ex Fr. on *P. strobus* (G); *Xylaria ianthinovelutina* Mont. on *Magnolia fraseri* (G).

DISCOMYCETES (sensu latu): *Aleuria rhenana* Fckl. (C, G); *Aleurina atrovinosa* (Cke.) Seaver (C); *Belonium carnulosum* Rehm (C); *Chlorociboria aeruginascens* (Nyl.) Kanouse (B, C); *C. versiformis* (Fr.) Seaver (C); *Chlorosplenium aeruginosum* (Oed.) De N. (O); *C. chlora* (Schw. ex Fr.) Mass. on *Castanea dentata* (G); *Coccomyces coronatus* (Schw. ex Fr.) Sacc. on *Rhododendron maximum* (G); *Colpoma azaleae* (Schw.) Cke. on *Azalea lutea* (G); *Dasyscypha nivea* Sacc. on *Quercus maxima* (G); on dead coniferous wood (B, C); *Dermea balsamea* (Pk.) Seaver on *Tsuga canadensis* (B, G); *Dermea puberula* Durand on *Vitis* (B); *Durandiella fraxini* (Schw.) Seaver on *Fraxinus americana* (G); *Galactinia michelii* Boud. (C); *Geoglossum glabrum* Pers. ex Fr. (C); *G. simile* Pk. (B); *Glonium abbreviatum* (Schw.) Lohman on *Quercus coccinea* (G); *Godronia rugosa* Ell. & Ev. on *Oxydendrum arboreum* (G); *G. urceolata* (Ell.) Hoehn. on *Clethra acuminata* (B, G); *Helotium caudatum*

(Karst.) Vel. on *Amelanchier laevis* (B, G); *H. virgultorum* (Vahl ex Fr.) Karst. on dead wood (B); *Humaria scutellata* (L. ex Fr.) Fckl. (G); *H. setosa* (Nees ex Fr.) Fckl. (B); *H. umbrorum* (Fr.) Fckl. (C); *Hyaloscypha atomaria* (Starb.) Nannf. (C); *Hypoderma commune* (Fr.) Duby on *Solidago boottii* (G); *Lachnum cerinum* (Pers. ex Fr.) Nannf. on *Cornus florida* (B, G); *L. ciliare* (Schrad. ex Fr.) Rehm on *Quercus* sp. (B); *L. spiraeaicolum* (Karst.) Rehm (C); *L. virgineum* (Batsch ex Fr.) Karst. (B, C); *Leotia lubrica* Scop. ex Fr. (C, O); *L. viscosa* Fr. (C); *Lophodermium nitens* Darker on *Pinus strobus* (G); *L. pinastri* (Schrad. ex Fr.) Chev. on *Pinus rigida* (G); *Macropodia macropus* (Pers. ex Fr.) Fckl. (B); *Mollisia caesia* (Fckl.) Sacc. (C); *Mollisia cinerea* (Batsch ex Fr.) Karst. (B, C); *Ocellaria ocellata* (Pers. ex Fr.) Schroet. on *Salix humilis, S. sericea* (G); *Orbilia curvatispora* Boud. (C); *O. inflatula* Karst. (B); *O. leucostigma* Fr. (B, C); *O. luteo-rubella* (Nyl.) Karst. (C); *O. xanthostigma* Fr. (B, C); *Pestalopezia rhododendri* Seaver on *Rhododendron maximum* (B, C, G); *Pezicula* sp. on *Oxydendrum arboreum* (G); *P. alnicola* Groves on *Alnus rugosa* (G); *P. carnea* (Cke. & Ell.) Rehm on *Acer rubrum* (B, G); *Peziza badia* Pers. ex Fr. (B, C, G); *Pezizella lythri* (Desm.) Shear and Dodge on *Galax aphylla* (B); *Propolidium glaucum* (Ell.) Sacc. on *Castanea dentata* (B); *Rhytisma acerinum* Pers. ex Fr. on *Acer rubrum* (B); *R. andromedae* Fr. (C); *R. decolorans* Fr. on *Xolisma* (*Lyonia*) *ligustrina* (B, G); *R. salicinum* Pers. ex Fr. on *Salix humilis* (G); *R. vaccinii* Pers. ex Fr. on *Polycodium melanocarpum* (B); *Rutstroemia macrospora* (Pk.) Kanouse (C); *Schizoxylon* sp. on *Cornus florida* (G); *Stictis quercifolia* Cke. on *Quercus maxima* (B, G); *Tapesia fusca* (Pers. ex Fr.) Fckl. (B); *T. riccia* (Sacc.) Rehm (C); *T. torulae* Fckl. (C); *Trichoglossum walteri* (Berk.) Durand (C, G); *Tympanis pithya* Karst. on *Pinus strobus* (G).

BASIDIOMYCETES:

Uredinales: Coleosporium helianthi (Schw.) Arth. on *Helianthus giganteus, H. tuberosus* (B, O); *C. inconspicuum* (Long) Hedgc. on *Coreopsis major* (B); *C. oemleri* (C); *C. solidaginis* (Schw.) Thuem. on *Solidago* sp. (O); *S. arguta* (B), *S. bicolor*

(B), *S. caesia* (G), *S. conferta* (G), *S. curtisii* (B), *S. monticola* (G), *S. rugosa* (G), *S. tomentosa* (B); *C. vernoniae* Berk. & Curt. on *Vernonia noveboracensis* (B, G); *Cronartium quercuum* (Berk.) Miy. on *Quercus coccinea* (G); *Frommea obtusa* (Str.) Arth. on *Potentilla canadensis* (G); *Gymnosporangium juniperi-virginianae* Schw. on *Juniperus virginiana* (O); *G. nidus-avis* Thaxt. on *J. virginiana* (O); *Kuehneola uredinis* (Lk.) Arth. on *Rubus argutus* (B, C, G); *Melampsora abieti-caprearum* Tub. on *Salix humilis* (B); *Puccinia angustata* Pk. on *Scirpus georgianus, S. sylvaticus* (G); *P. bolleyana* Sacc. on *Carex lurida* (G); *P. coronata* on *Phleum pratense* (G); *P. helianthi* Schw. on *Helianthus tomentosus* (G); *P. menthae* Pers. on *Koellia beadlei* (G); *P. mesomajalis* Berk. & Curt. on *Clintonia borealis* (B, C); *P. rubigo-vera* (DC.) Wint. var. *impatientis* (Arth.) Mains on *Agrostis perennans* (G); *P. smilacis* Schw. on *Smilax* sp. (B); *P. tenuis* (Schw.) Burr. on *Eupatorium urticaefolium* (B); *P. violae* (Schum.) DC. on *Viola primulifolia* (B, G); *Pucciniastrum hydrangeae* (Berk. & Curt.) Arth. on *Hydrangea arborescens* (B, C, G, O), *H. cinerea* (B); *P. myrtilli* (Schum.) Arth. on *Azalea* sp. (B, C, O); *Uredinopsis macrosperma* (Cke.) Magn. on *Pteridium aquilinum* (O); *Uromyces hedysari-paniculati* (Schw.) Farl. on *Desmodium canescens* (B, C, G).

TREMELLALES: *Calocera cornea* (Fr.) Lk. on *Rhododendron maximum* (B); *Dacrymyces minor* Pk. (O); *D. palmatus* (Schw.) Bres. (O); *Exidia nucleata* (Schw.) Burt (O); *E. recisa* (S. F. Gray) Fr. (O); *Guepinia spathularia* (Schw.) Fr. (O); *Sebacina incrustans* Pers. ex Tul. (B); *S. podlachica* Bres. (O); *Tremellodendron candidum* (Schw.) Atk. (B, O); *T. merismatoides* (Schw.) Burt (B, O); *T. tenax* (Schw.) Burt (O); *Tremellodon gelatinosum* (Pers.) Fr. (O).

HOMOBASIDIOMYCETES (ex parte, see also the following paragraphs): *Aleurodiscus candidus* (Schw. ex Fr.) Burt on *Quercus alba, Q. borealis* (B); *Amanita caesarea* Fr. (B, O); *A. muscaria* L. ex Fr. (O); *A. rubescens* Fr. (B); *A. solitaria* Fr. (S); *Armillaria aurantia* Fr. (S); *Calodon geogenium* (Fr.) Karst. (B, S); *C. scrobiculatum* (Fr.) Quél. (B); *C. velutinum* (Fr.) Quél. (S); *C. zonatum* (Batsch ex Fr.) Quél. (B); *Cantharellus floccosus*

Schw. (B); *Clavaria mucida* Pers. (O); *Clitocybe clavipes* Fr. (B); *C. illudens* Schw. (B); *Corticium roseum* Pers. ex Fr. on *Populus tremuloides* (G); *Craterellus cornucopioides* L. ex Fr. (B); *Cyclomyces greenei* Berk. (C); *Dentinum albidum* (Pk.) Snell (S); *D. repandum* (L. ex Fr.) S. F. Gray (S); *Fistulina hepatica* Huds. ex Fr. (B); *Fomes applanatus* (Pers. ex Fr.) Gill. (B); *Hydnum fennicum* (Karst.) Sacc. (B, S); *Hymenochaete arida* Karst. on *Cornus florida* (B); *H. corrugata* (Fr.) Lév. on *Acer rubrum* (B); *H. fuliginosa* (Pers.) Bres. on *Rhododendron maximum* (B); *Lactarius subpurpureus* Pk. (S); *L. volemus* Fr. (S); *Lenzites betulina* L. ex Fr. (B); *L. saepiaria* Wulf. ex Fr. on *Tsuga canadensis* (B); *Marasmius* sp. on *Tsuga canadensis* (B); *Panus stypticus* Fr. on *Rhododendron maximum* (B); *Peniophora sanguinea* (Fr.) Bres. on *Alnus* (B); *P. subalba* (Burt) Rogers & Jackson on *Alnus* (B); *Pholiota caperata* Fr. (S); *Phylloporus rhodoxanthus* (Schw.) Bres. (S); *Polyporus abietinus* Dicks. ex Fr. on *Picea rubens* (G); *P. adustus* Willd. ex Fr. on *Acer rubrum* (G); *P. berkeleyi* Fr. (B); *P. cinnabarinus* Jacq. ex Fr. (B); *P. cinnamomeus* Jacq. ex Fr. (B, S); *P. elegans* Bull. ex Fr. (B); *P. focicola* Berk. & Curt. (S); *P. giganteus* Pers. ex Fr. (B, G); *P. hirsutus* Wulf. ex Fr. (B, O); *P. pargamenus* Fr. on *Acer rubrum* (B); *P. perennis* L. ex Fr. (C); *P. sulphureus* Bull. ex Fr. (C); *P. tulipiferus* (Schw.) Overh. on *Castanea dentata* (B); *P. versicolor* L. ex Fr. on *Populus tremuloides* (G), *Rhododendron maximum* (B), *Sassafras officinale* (B), dead wood (O); *Poria* sp. on *Acer rubrum* (G); *P. isabellina* (Fr.) Overh. on *Rhododendron maximum* (B); *P. versipora* (Pers. ex Fr.) Romell on *R. maximum* (B); *Russula crustosa* Pk. (S); *R. emetica* Fr. (O); *R. foetans* Fr. (S); *R. variata* Bann. & Pk. (S); *R. virescens* Fr. (S); *Schizophyllum commune* Fr. (O); *Solenia anomala* (Pers. ex Fr.) Fckl. on *Betula lutea* (G); *S. fasciculata* Pers. on *Alnus* (B); *Steccherinum ochraceum* Pers. ex S. F. Gray on *Castanea dentata* (B); *Stereum frustulatum* (Pers. ex Fr.) Fr. on *Quercus* sp. (G); *S. hirsutum* Willd. ex Fr. on *Acer rubrum* (B); *S. lobatum* (Kunze) Fr. (O); *S. rameale* Schw. on *Nyssa sylvatica* (B), *Rhododendron maximum* (B), dead wood (O); *Thelephora vialis* Schw. (S); *Tomentella coriaria*

(Pk.) Bourd. & Galz. on *Rhododendron maximum* (B); *Tricholoma equestre* Fr. (S).

BOLETACEAE (All collections by Dr. W. H. Snell unless otherwise indicated): *Boletellus betula* (Schw.) Gilbert; *Boletinus pictus* Pk. (B, S); *Boletus bicolor* Pk.; *B. edulis* Bull. ex Fr.; *B. ferrugineus* Frost; *B. fumosipes* Pk.; *B. griseus* Frost; *B. illudens*; *B. miniato-olivaceus* Frost; *B. modestus* Pk.; *B. nobilis* Pk.; *B. peckii* Frost; *B. separans* Pk.; *B. vermiculosus* Pk.; *Gyroporus castaneus* (Bull. ex Fr.) Quél.; *Leccinum chromapes* (Frost) Singer; *L. nigrescens* (Rich. & Roze) Singer; *L. rubropunctum* (Pk.) Singer; *L. rugosiceps* (Pk.) Singer; *L. scabrum* (Bull. ex Fr.) S. F. Gray; *Porphyrellus gracilis* (Pk.) Singer; *Pulveroboletus auriflammeus* (Berk. & Curt.) Singer; *P. auriporus* (Pk.) Singer; *P. ravenelii* (Berk. & Curt.) Singer; *P. retipes* (Berk. & Curt.) Singer (B, S); *Strobilomyces floccopus* (Vahl ex Fr.) Karst. (O, S); *Suillus granulatus* (L. ex Fr.) Kuntze ssp. *snellii* Singer; *S. punctipes* (Pk.) Singer; *S. subaureus* (Pk.) Snell; *Tylopilus alboater* (Schw.) Murr.; *T. eximius* (Pk.) Singer; *T. felleus* (Bull. ex Fr.) Karst.; *Xanthoconium affine* (Pk.) Singer; *Xerocomus subtomentosus* (L. ex Fr.) Quél. From near Winston-Salem, No. Car.: *Strobilomyces confusus* Singer, *Tylopilus felleus* var. *minor* Coker & Beers.

GASTEROMYCETES: *Calostoma cinnabarina* Desv. (B); *Crucibulum vulgare* Tul. (B); *Lycoperdon atropurpureum* Vitt. (B); *L. echinatum* Pers. (C); *L. gemmatum* Batsch (B); *L. pyriforme* Schaeff. (O); *L. subincarnatum* Pk. (B); *Scleroderma geaster* Fr. (S); *S. lycoperdoides* (Schw.) (C).

FUNGI IMPERFECTI: *Aegerita epixylon* DC. ex Duby on *Castanea dentata* (B); *Cercospora dioscoreae* Ell. & G. Martin on *Dioscorea villosa* (G); *C. omphacodes* Ell. & Holw. on *Phlox* sp. (B); *C. rubi* Sacc. on *Rubus flagellaris* (G); *C. smilacina* Sacc. on *Smilax bona-nox* (B); *C. smilacis* Thuem. on *Smilax glauca* (B, G); *C. venturioides* Pk. on *Asclepias syriaca* (G); *C. vexans* C. Massal. on *Fragaria virginiana* (G); *Chondropodiella clethrincola* (Ell.) Hoehn. on *Clethra acuminata* (B, G); *Cladosporium epiphyllum* Pers. ex Fr. on *Robinia pseudoacacia* (G); *C. gloeosporioides* Atk. on *Hypericum mutilum* (G); *Colletotrichum trichellum* (Fr.)

Duke on *Hedera helix* (B); *Cylindrosporium acerinum* Tracy & Earle on *Acer pennsylvanicum* (B); *Cytospora pinastri* Fr. on *Abies fraseri* (G); *Darluca filum* (Biv.) Cast. on *Coleosporium solidaginis* and other Uredinales (B, C); *Discosia artocreas* (Tode ex Fr.) Fr. on *Epigaea repens* (B); *Glocosporium caryae* Ell. & Dearn. on *Carya glabra* (G); *Graphium hamamelidis* Van Hook on *Hamamelis virginiana* (B, G); *Helminthosporium* sp. on *Phleum pratense* (C); *Hendersonia heloniaefolia* (Cke. & Ell.) Cke. on *Helonias* sp. (B); *Isariopsis clavispora* (Berk. & Curt.) Sacc. on *Vitis bicolor* and *Vitis* sp. (B, G); *Macrophoma smilacis* (Pk.) Berl. & Vogl. on *Smilax glauca* (G); *Monochaetia desmazierii* Sacc. on *Castanea dentata* (B); *Monotospora megalospora* Berk. & Br. on *Castanea dentata* (B); *Penicillium camemberti* Thom on agaric (B); *Penicillium thomii* Maire (C); *Pestalotia funerea* Desm. on *Thuya occidentalis* (G); *Phyllosticta epigaeae* Pk. on *Epigaea repens* (B); *Sepedonium brunneum* Pk. (C); *Sepedonium chrysospermum* (Bull.) Lk. ex Fr. (B); *Septocylindrium concomitans* (Ell. & Holw.) Halst. on *Bidens frondosa* (G); *Septoria cornicola* Desm. on *Cornus alternifolia* (G); *S. violae* West. on *Viola primulifolia* (B, G); *Septoria* sp. on *Zanthorhiza apiifolia* (G); *Sphaceloma plantaginis* Jenkins & Bitanc. on *Plantago rugelii* (B); *Sphaerographium* sp. on *Azalea arborescens* (G); *Sporocybe azaleae* (Pk.) Sacc. on *Rhododendron maximum* (B, C); *Sporodesmium antiquum* Cda. (B); *Sporotrichum quercuum* Shear on *Quercus* sp. (B); *Stilbella ostracogena* Cda. on *Castanea dentata* (B); *Volutella* sp. on *Magnolia fraseri* (G).—JOHN A. STEVENSON.

PLANT INDUSTRY STATION,
BELTSVILLE, MARYLAND

PROPOSALS FOR AMENDMENT OF THE INTERNATIONAL RULES

I

Art. 47. An alteration of the diagnostic characters or of the circumscription of a group **without exclusion of the type** does not warrant the citation of an author other than the one who first published its name.

Proposal: delete the words "without exclusion of the type."

Argument: The sentence quoted is the first and essential sentence in Art. 47 as amended at Amsterdam (Bisby, G. A., An introduction to the taxonomy and nomenclature of fungi, p. 81. 1945; Brittonia **6**: 16. 1947). The phrase in bold-face is the modification (Zesde Int. Bot. Congr. Amsterdam Proc. **1**: 344. 1936). It, and with it the whole sentence, is ambiguous in the extreme. If the sentence means anything, it implies the affirmative statement: **An alteration of the diagnostic characters or of the circumscription of a group with exclusion of the type warrants the citation of an author other than the one who first published the name.** Since "a nomenclatorial type is that constituent element of a group to which the name is permanently attached" (Art. 18), such exclusion would seem to be excluded by the definition of "type." Under such an interpretation, which is the logical one, the added phrase is inoperative and achieves no end save that of confusion, and clearly should be revoked. But it is likely that the implications of the type concept will be overlooked and the amended article illogically accepted, although often under protest, as implying the positive paraphrase just given.

The author of a name is cited "for the indication of the name of the group to be accurate and complete" (Art. 46). After the incorporation of the type concept into the rules, and before the addition of the phrase under discussion, the author was the one "who first published the name in question" (Art. 46). Knowing the name and its author, a taxonomist was at least well on the way to learning the date of the name (for consideration of priority), and the type, or at least, the constituent elements eligible as lectotype (for determining the application of the name). This is not to say that either could always be "readily verified"; but at least the field of investigation was greatly narrowed. Homonyms could give rise to difficulties; but a straightforward and honest homonym could be dealt with by the "Auct. II non Auct. I" formula widely used long before it was adopted as Rec. XXXII bis (Bisby, l.c., p. 83; Brittonia **6**: 18; Amsterdam Proc. **1**: 344); homonyms were identical names independently published for different types.

But under the amended Art. 47, according to the interpretation

under discussion, there are as many homonyms of any name as there are uses of that name with "alteration of the diagnostic characters or of the circumscription of a group [with] exclusion of the type." The first two points are easy to identify. Since a later author rarely repeats exactly in his description the original characters ascribed to a group, a majority of subsequent descriptions embody "alteration of the diagnostic characters." One need scan the successive descriptions of an early genus in only a few examples to make sure of that: *Agaricus* L. ex Fr. was first validly published with "lamellae . . . ascigerae," but later described as possessing basidia, and most recently as annulate and with purple-brown spores. *Puccinia* was first validly described as having spores "septulis distinctis"; additional characters have repeatedly been added, as that the spores are one-septate (excluding multiseptate species) and that the telia are dry (excluding those with gelatinous horns). Since a later author usually adds to or subtracts from the number of original species, either incidentally or systematically, the majority of subsequent treatments embody "alteration of the . . . circumscription of a group." *Agaricus* has been both repeatedly enlarged, by the addition of new species, and repeatedly narrowed, by exclusion of one component after another as autonomous genera, and finally has been restricted to Ser. *Pratella* Trib. *Psalliota*. *Puccinia* has similarly been enlarged, and similarly narrowed by the exclusion of *Phragmidium, Gymnosporangium, Tranzschelia,* etc. It is clear that such alterations of the diagnostic characters and circumscription do not warrant the citation of an author other than the one who first published the name—unless (according to the interpretation under discussion) accompanied by exclusion of the type.

Assuming without prejudice that the type can be excluded, how can it be done? It is here that the sentence under discussion is completely ambiguous. There are many possible ways of excluding the type: 1. By description. Clements & Shear described *Tulasnella* (in a key: Gen. Fung. 158) as having "basidia . . . cruciately divided by 3 vertical septa," thus excluding *T. lilacina,* the genotype. 2. By a statement of geographic range which excludes the type species or specimen. 3. By designation of another type. Clements & Shear designated *T. anceps,* an alien species, as

the type of *Tulasnella* (l.c., p. 342). 4. By exclusion of the type species (etc.) from the subgenus (etc.) named *Typicae*, *Eu-*, or otherwise marked as the typical subdivision. Saccardo (Syll. **4**: 283–288) excluded both original species of *Zygodesmus* from I. *Eu-Zygodesmus*, assigning them to II. *Hypochniella* B. *Leiosporae* of *Zygodesmus*. 5. By relegating the type to a list of *Ignotae*, *Dubiae*, etc., having adequately described other components of the group. 6. By various other phrases, comments, or discussions indicating that the characters and circumscription in a particular treatment were derived chiefly from other elements of the group than the type. 7. By omission of the type. When an author discusses a genus as it occurs in Italy, he omits its type if that occurs only in Germany. When an author enumerates material of a species collected in South America, he may omit its type if that was collected in the Caribbean. 8. By assignment or transfer of the type to another group. Desmazières wrote (Ann. Sci. Nat. iii **11**: 279): "Le *Phoma pustulata*, Fr., [an erroneous spelling, but undoubtedly the designated type of *Phoma*] est un *Sphaeria*." Sparrow (Aquatic Phycomycetes) assigned all 16 original species of *Phlyctidium* to other genera. Saccardo (Syll. **2**: 285) transferred both original species of *Venturia* to *Pyrenophora*. Each of these authors nevertheless used the generic name in question for other species.

It is unlikely that any author would pounce upon all these possibilities as constituting veritable exclusion of the type, and likely that most taxonomists, if they allowed the possibility that the type could be excluded, would accept only the last: explicit exclusion, or explicit assignment of the type to another group. Nevertheless, since none of them is eliminated by Art. 47, nor by any other rule (unless all are eliminated by Art. 18), Art. 47 is at present insuperably ambiguous.

Supposing the interpretation under consideration to be the accepted one, what are the effects of the interpolation "without exclusion of the type"? They are two: 1. The bringing into existence of a number of validly published homonymous groups where only one stood before. The number depends, of course, on what is held to constitute exclusion of the type. Such groups will clutter up the synonymy, and greatly increase the labors of tax-

onomists, but can and will be disregarded by most. 2. The creation of the possibility of recognizing a name *in any sense in which it has been misapplied* (if exclusion of the type can be plausibly argued) and, if the name is that of a genus, of conserving it. That seems to be the one use of the amendment and all the trouble it entails, and the one occasion for its adoption: a device to permit the retention, by a change in author, of generic names which have been incorrectly used. It is one more evasion of the type concept, which has proved so useful to many botanists and so distasteful to a few. As such it is burdensome, confusing, and inaccurate. The modification is "on trial until the next Congress" (Art. 74), which should reject it. Art. 18 contains the sound and practical provision for such cases: "The name of a group must be changed if the type of that name is excluded."

II

Proposal: To adopt, as a part of Appendix I, the following

REGULATIONS FOR FIXING GENERIC TYPES

I. RULES

1. The type species shall be the species or one of the species included in the genus when first validly published after the applicable date set forth in Art. 20. If a genus included but one species when originally published, that species is the type. The types of genera adopted through citations of literature published before the starting-point for the group in question (with or without change of name) shall be selected from those of the original species which received names in the first legitimate publication.

2. When, in the original publication of a genus, one of the species was definitely designated as type, that species shall be accepted as the type, regardless of other considerations. If *typicus* or *typus* was used as a new specific name for one of the species, that species shall be accepted as the type as if it were definitely designated.

3. The publication of a new generic name as an avowed substitute for an earlier one does not change the type of the genus.

4. If a genus without an originally designated type contains among its original species one with the generic name used as a specific name, either as an accepted name or synonym, that species is to be accepted as the type.

5. If a genus when originally published included more than one species, and no species was definitely designated as type, nor indicated according to Paragraph 4, the choice of the type shall accord with the following principles:

(a) Species inquirendae or species doubtfully referred to the genus or mentioned as in any way exceptional are to be excluded from consideration in selecting the type.

(b) Species which definitely disagree with the generic description (provided others agree), or which possess characters stated in the generic description as rare or unusual, are to be excluded from consideration in selecting the type.

6. Among species equally eligible, the preference shall be given to the first known to have been designated as the type.

II. RECOMMENDATIONS

In the selection of types of genera previously published, but of which the type would not be indicated by the preceding regulations, the following points are to be taken into consideration:

i. The type species should usually be the species or one of the species which the author had chiefly in mind. This is often indicated by

(a) A closer agreement with the generic description.

(b) Certain species being figured (in the same work).

(c) The specific name, such as *vulgaris, communis, medicinalis* or *officinalis*.

ii. The type species should usually be the one best known to the author. It may be assumed that an indigenous species (from the standpoint of the author), or an economic species, or one grown in a botanical garden and examined by the author, would usually represent an author's idea of a genus.

iii. The preceding conditions having been met, preference should be shown for a species which will retain the generic name in its most widely used sense, or for one which belongs to a division of

the genus containing a larger number of species, or for the historically oldest species.

iv. If it is impossible to select a type under the conditions mentioned above the first of equally eligible species should be chosen.

Discussion: The present Rules are somewhat prolix in their provisions for determining the proper names for plants, but quite vague and unsatisfactory in indicating for what plants these names are to be used. The **type method** was written into the Rules (Art. 18; Rec. IV–VII) in response to the demand for regulations covering the applications of names; but perhaps out of fear that it would invalidate current irregular or perverted usages, Appendix I, "Regulations for determining types," has not been written. In fact, the heading for this Appendix bears the note "No draft of this Appendix has been submitted."

Whether or not such a draft has been formally "submitted," such drafts, and excellent ones, have been prepared and published. The one here proposed is essentially a rearrangement of one written by a committee of the Botanical Society of America, composed of A. S. Hitchcock (Chairman), N. L. Britton, and J. M. Greenman, with the collaboration of Leroy Abrams and Witmer Stone, and published in Science **49**: 333–335. 1919. It has the advantages of being clear, simple, and honest, and the further advantage of embodying established custom (Art. 5)—that is, the consistent practice of most botanists (and of nearly all botanists when they are not seeking justification for the employment of a beloved, but illegitimate nomenclature). If nomenclature is to be governed by rules rather than exception, Appendix I should no longer be left a blank; and these provisions recommend themselves as filling a pressing need.—DONALD P. ROGERS.

NEW YORK BOTANICAL GARDEN

SPECIAL COMMITTEE ON FUNGI FOR THE INTERNATIONAL BOTANICAL CONGRESS AT STOCKHOLM.—At the International Conference on Botanical Nomenclature held at Utrecht, Netherlands, in June 1948, additional members were added to the special committee on fungi in order to broaden and expedite the work necessary to prepare for the Stockholm Congress. New appointees were J. Westerdijk (Centraalbureau v. Schimmelcultures, Baarn,

Netherlands), D. P. Rogers (N. Y. Bot. Gard., Bronx Park, New York, N. Y.), C. W. Emmons (Nat. Inst. Health, Bethesda, Md.), G. W. Martin (Univ. Iowa, Iowa City, Iowa), R. Singer (Inst. Miguel Lillo, Calle Miguel Lillo, Tucuman, Argentina), S. P. Wiltshire (Commonwealth Mycol. Inst. Kew, Surrey, England), M. Le Gal (6 Rue Chomel, Paris VI, France), M. A. Donk (Herb. & Mus. Syst. Bot., Buitenzorg, Java), and B. B. Mundkur (Indian Agr. Res. Inst., New Delhi, India). Previously appointed members of this committee are C. L. Shear (2253 No. Madison St., Arlington, Va.), K. B. Boedijn (Herb. & Mus. Syst. Bot., Buitenzorg, Java), R. Ciferri (Bot. Inst. & Ital. Crypt. Lab., Univ. Pavia, Pavia, Italy), R. Maire (3 Rue Linné, Algiers, Algeria), J. A. Nannfeldt (Uppsala Univ. Inst. Syst. Bot., Uppsala, Sweden), F. Petrak (Naturhist. Mus., Wien I, Burgring 7, Austria), F. J. Seaver (N. Y. Bot. Gard., Bronx Park, New York, N. Y.), E. M. Wakefield (Royal Bot. Gard., Kew, Surrey, England), A. M. Bottomley (Dept. Agr. Pretoria, Union S. Africa), W. J. Lütjeharms (Univ. Coll., O. F. S., Bloemfontein, Union S. Africa), J. Ramsbottom (Brit. Mus., Cromwell Road, London, S. W. 7, England), A. Trotter (R. Ist. Super. Agr. Portici, Naples, Italy), and W. H. Weston (Harvard Univ., Divinity Ave., Cambridge, Mass.). T. A. Sprague (care of Royal Bot. Gardens, Kew, Surrey, England), and J. Lanjouw (Bot. Mus. & Herb., 106 Lange Niewstraat, Utrecht, Netherlands) are "ex officio" members of all committees.

It is the duty of this committee to consider all proposals to modify in any way the present International Rules of Botanical Nomenclature in so far as fungus nomenclature is concerned. Each specific proposition should be submitted with at least two copies to each member of the committee. Each member will carefully consider each proposal and indicate clearly whether for or against it and return one marked copy to the secretary as promptly as possible in order that the results may be compiled and made ready for the Congress. It is hoped that this notice will be accepted by committee members in lieu of a personal letter and that we may have the fullest cooperation in carrying out the work.—C. L. SHEAR, Secretary, 2253 No. Madison St., Arlington, Virginia, U. S. A.

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